Influence of Flour and Processing Factors on Flavor Attributes of Extruded Corn Puffs

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Dedication

To my family and friends who have supported me tirelessly through my graduate school career and especially over the previous year when I needed it most.

Abstract

Consumer awareness regarding the impact of nutrition on health and wellness has been steadily growing, along with interest in fresh, natural and wholesome foods and ingredients. Despite the growing demand for healthy food, and the increasing information supporting the contribution of whole grain products to a healthy diet consumers still fail to meet the USDA recommended daily amount. Among the major factors limiting consumption of whole grains are negative sensory attributes associated with whole grain products. When cereal foods are manufactured with whole grain instead of refined grain flour, lower product acceptability is observed.

Understanding how whole grains differ from more widely accepted refined grain flours, and the mechanisms of whole grain flavor development is a vital step in improving the food production system to meet consumer flavor expectations. To address flavor quality challenges, this work had two distinct yet, related goals ultimately aiming to improve the flavor profile of extruded whole grain products. The first aimed to characterize the key aroma differences between refined and whole grain extruded corn puffs. The second goal aimed to understand how processing can influence the content and delivery of bitter compounds from the food matrix. Both aimed at gaining insight on how ingredients and processing affect flavor development and perception thus, providing a basis for food producers to tailor extrusion processing to create more palatable wholesome products and boost consumer acceptance.

Key aroma compounds that drive the differences in the refined and whole grain extruded corn puffs were characterized to better understand the flavor performance of

whole grain formulated corn puffs and related mechanistic pathways of flavor generation. Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) and aroma extract dilution analysis (AEDA) were utilized to examine aroma profiles and identify key aroma differences between whole grain and refined grain extruded corn puffs. Following identification sensory descriptive analysis was performed to evaluate the impact of compounds identified through AEDA on aroma attributes of the corn puffs.

GC-O/MS and AEDA analysis identified 13 odor active compounds with different aroma intensity and quantitative concentrations between the whole grain and refined corn puffs. Of the identified aroma compounds 9 were significantly higher in concentration in the whole grain puffs (WGP), 2-methyl-pyrazine, 2,3-dimethylpyrazine, methylthiazoline, 2-ethyl-3,5-dimethylpyrazine, 3-hydroxy-2-methyl4H-pyran-4-one, 2-methoxyphenol, 2-acetyl-2-thiazoline, 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde were higher in concentration in while one compound 2,5-dimethylpyrazine was higher in concentration in refined grain puffs (RFP). Based on the chemical structures of the identified odorants three reaction pathways that drive aroma differences were identified as of interest; the Maillard or non-enzymatic, phenolic degradation and the lipid oxidation reaction pathways.

Descriptive Analysis (DA) was employed to investigate the sensory impact of the identified odorants important for aroma attribute differences. In general, the results indicated that the perceived puffed aroma intensities of the RFP and WGP profiles agreed with the quantitative analysis of the identified aroma compounds. The WG puffs were reported to have a greater intensity of cooked, corn chip, roasted and toasted attributes.

Which was reflective of the higher concentration of Maillard reaction products which have aroma characteristics like roasted and toasted

In addition to changes in the aroma attribute intensities, other flavors characteristics like taste are important. One bitter compound chaenorpine, a major contributor of bitterness in extruded WGP, was also examined. The impact of processing treatments applied to flour, addition/exclusion of ingredient aids, pH modification and flour aging, on the concentration of available chaenorpine were explored.

The influence of heat treatment on bitterness generation was examined using both a bench scale thermal processing protocol, as well as pilot scale extrusion. Chaenorpine concentration was monitored via LC/MS/MS and was determined in solvent extracts, reflecting total content as well as in saliva extracts after mastication, reflecting the oral concentration during consumption. The results indicated that addition of tri-sodium phosphate (TSP) reduced the level of chaenorpine in the puffs. The observed reduction was thought to be related to the ability of the processing aid to raise the pH of the sample. A series of pH modifications were further examined the influence of pH on chaenorpine content. The results revealed a two-fold reduction of chaenorpine when pH of the sample was in the basic region when compared to acidic.

Sensory studies agreed with analytical findings and confirmed that the overall perception of bitterness was reduced in puffs, when TSP was included in the formulation and the concentration of chaenorpine was reduced. Thus, providing a potential ingredient method that could effectively reduce bitterness perception of whole grain corn products and improve the overall flavor profile.

In summary, this work illustrated the significant impact of inherent flour chemistry on the development of the flavor profile of extruded corn products and related pathways of flavor generation. Understanding aroma and bitterness development provides the foundation for processing and formulation strategies for flavor optimization ultimately to increase consumer acceptability and consumption.

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Chapter 1: Literature Review

Flavor Overview

Senses allow living organisms to interact with the environment, gain information and make decisions. There are five basic senses; sight, sound, touch, taste and smell that gather environmental information, that is relayed to the brain and used to generate internal responses. One experience where senses meld together is eating. How appalling or appealing a food seems is influenced by all the senses. While things like the perceived appearance, texture and sounds are associated with eating, the chemical senses of taste and smell are major drivers of the experience. Ultimately, the experience results in acceptance and preference (2).

Aroma and taste are the two primary drivers of flavor perception. Aroma is experienced through the sensation of smell and is the result of sensing many compounds. To date there have been over 1000 detectable aroma compounds identified in many different foods (3). The compounds come from several chemical classes and have a broad range of thresholds. Aroma compounds are detected through ortho-nasal channels, through the nostrils, or retro-nasal channels, through the back of the mouth during mastication and reaching the odor receptors. The reaction responsible for producing aroma compounds will be discussed below.

Taste is detected by the tongue through taste buds. In every individual taste bud, there are between 50 and100 taste receptor cells. The receptor cells when triggered by chemical stimuli generate responses that are transferred through the gustatory system or taste sensory system as neural information to the brain where the sensations are

experienced. Taste has five primary qualities including sweet, sour, salty, bitterness and the more recently understood umami, a savory taste. While considerably less taste active compounds have been identified to date, taste is no less complex in analysis than aroma. The intricacies of the analysis will be discussed below.

Both aroma and taste should be thought branches of the same tree, both are important factors in the sensory experiences that are derived from food and eating. Interestingly the sensory experience is also influenced by several factors including sensory inputs from chemesthetic sensations such as touch, thermal perception as well as auditory and visual ques (4).

Flavor perception is a key driver of consumer acceptance which, ultimately shapes eating habits and has the potential to greatly impact health and disease prevention through diet. Thus, providing practical insight for flavor quality improvement of healthy foods can assist consumers make better food choices.

Health Benefits of Whole Grains

Whole grains offer a host of health benefits in what has been called the "whole grain package" (5). Recently, it was demonstrated that children who consume whole grain breakfast cereal are more likely to have a healthy body weight compared to children who choose other breakfast options or skip breakfast entirely (6). Whole grain consumption was also found to regulate diabetes via regulation of blood glucose levels (6,7). Studies also linked consumption of whole grains to lower rates of cancers of the stomach (8), mouth and digestive tract (9) and endometrial tissue (10). Lastly

epidemiological studies have linked consumption of whole grains to a lower risk of coronary heart disease, a leading cause of death domestically (11).

In addition to the above health benefits, whole grains contain a wide variety of wholesome nutrients that are important for overall wellbeing such as amino acids, minerals, polyphenols and a wide variety of essential vitamins such as thiamin, niacin, riboflavin and pantothenic acid. Whole grains are also recognized source of soluble and insoluble fiber, which have been shown to provide beneficial health effects. Soluble fiber has been linked to decreased cholesterol while insoluble fiber has been shown to improve laxation and increase feelings of satiety. Despite the overwhelming body evidence of the benefits of whole grains, and the increase consumer awareness regarding the health benefits of whole grains consumption remains low.

The USDA guidelines recommend2000 calorie diet have 6 ounces of grains per day with 50% being whole grains (12). Despite this it is estimated that only about 5% of the population of the United States is consuming the recommended amount. Many factors contribute to the observed gap between recommended and actual dietary intake, including flavor challenges, consumer's difficulty in identifying whole grain products, limited availability in certain markets and/or cost.

Hurdles associated with cost to the consumer and identifying foods that are whole grain products are concerns that can be addressed through channels such as public health, education, or advertising. Nonetheless, achieving a higher flavor quality is still a challenge for the producers and negative sensory attributes remain the primary reason for the lower consumption of whole grains. Simply put consumers, will not choose goods

that do not meet their taste preferences (9). Understanding how processing affects the flavor generation of whole grains can aid the development of optimized processing methods and product formulation approaches that result in better tasting products from a wide variety of whole grains. Corn is one of the most widely grown crops worldwide, and domestically is the most grown crop, responsible for approximately a quarter of the acres of crops grown in the United States (13). It is not surprising that major cereal producers, offer a range of corn-based cereals. While corn is not the only grain used for cereal production, it does have notable nutritional qualities as it has low fat content and more than double the fiber of any other major grain (Table 1.1) making it a great food ingredient for increasing dietary fiber intake. This is particularly important as; despite the known health benefits grain, consumption in the United States is very low with only 50% of Americans consuming the recommended daily amount of fiber (14).

	Corn	**Wheat**	**Oat**	**Rice**
Energy (kcal)	224	216	246	316
Protein (g)	8.36	15.55	17.3	13.35
Total lipid (g)	0.92	4.25	7.03	20.85
Fiber (g)	79	42.8	15.4	21
Sugars (total, g)	0	0.41	1.45	0.9

Table 1.1: Nutrients in 100 g of whole grain corn, wheat, oat and rice (11)

The Cereal Market Today and a Brief History

Ready-to-Eat (RTE) breakfast cereal makes up a sizable segment of the food market. The market for breakfast cereal was tracked by the Mintel Group from 2008-2011 (Figure 1.1). In 2011, the market was domestically worth an estimated $11.9 billion USD with RTE cereal comprising 88% of that market (14). It is also noteworthy that 96% of children ages 6- 11 regularly consume RTE cereal (15). Due to the wide spread consumption, RTE breakfast cereals reach a large population and serve as a major platform for promoting and ensuring whole grain consumption.

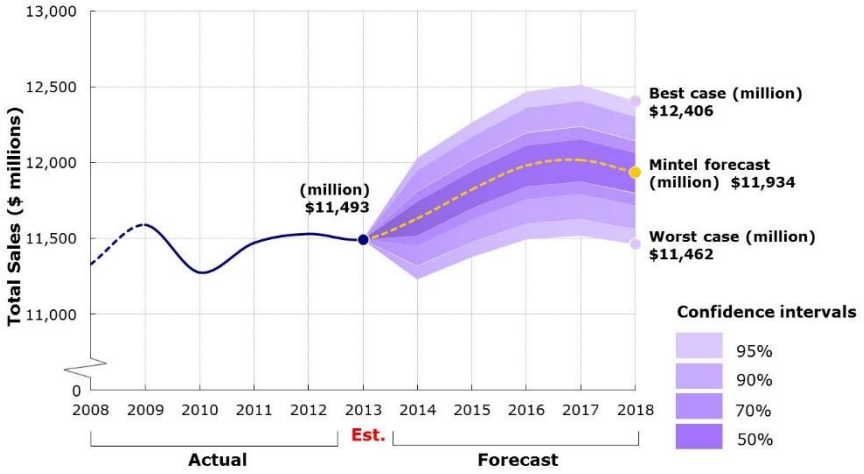


Figure 1.1: Breakfast Cereal Market Sales and Projected Growth (15)

Grains are a unique case as they have been grown and evolved with humans, as have the processing techniques. Refined grain products historically have been reserved to the rich for consumption. The reason for this is refined flour was difficult to produce due to the much more intensive milling required to produce the tastier if less nutritious grain. With the rise of industrial milling, new techniques emerged to produce refined flour at a much more rapid and cost effective rate. Interestingly this is about the time that whole

grain promotion began with individuals like Sylvester Graham, the inventor of the graham cracker. One way that whole grains was promoted was through early forms of ready to eat breakfast cereal.

Ready to eat breakfast's history began in 1863 with Dr. James Caleb Jackson pushing the consumption of whole grains (17,18). Jackson, a staunch vegetarian and firm believer in health promotion through food, owned and operated the Dansville Sanitarium. Jackson's business offered services aimed at increasing the well-being of customers through dietary intervention. Jackson's patrons consumed Granula, the first ready to eat breakfast cereal. Granula consisted of nuggets of bran soaked overnight rendering them chewy but still hard in the center, and were noted as being unpleasant due to texture. A patron of Jackson's institute, Ellen G. White, the founder of the Seventh Day Adventists, indirectly pushed ready to eat cereal further. White inspired one of her church's members, a physician, Dr. John Kellogg to improve the food.

Kellogg was considered by many to be a skilled surgeon with an obsession for bowel care. Armed with a desire to provide patient with a food that could help ease bowel ailments that were common at the time Kellogg designed his own Granula. Kellogg's Granula was a mixture of ground oats, corn and wheat, all ingredients incorporated in modern cereal. Because of a lawsuit filed by Jackson claiming copyright infringement Kellogg's Granula became known as granola. Kellogg's innovation would not stop here. With the help of the sanitarium accountant and his brother Will Kellogg, John Kellogg would design a new version of breakfast cereal and through a twist of fate would create a very recognizable addition to pantries across America.

The new iteration of breakfast was prepared using wheat meal. First the wheat meal was boiled then subjected to rollers. The rollers created a very thin cracker like sheet that was then roasted. One fateful night the brothers would leave a tray of boiled wheat berries on a counter. Upon discovering the berries the next day the brothers in a desire to minimize waste, ran the berries through the rollers. To their surprise the berries produced individual flakes. The newly created medical food was so loved by patients, that many following their return home would write to the Kellogg brothers asking for their own supply. Will would create corn flakes and would later go on to successfully market Corn Flakes. In 1906, the Kellogg company was founded and remains one of the modern-day bastions of ready to eat breakfast cereal.

Like many products, the breakfast cereal quickly saw an explosion in competition. Some of the first competitors for the Kellogg Company were the C.W. Post Company. Post's products would include a wheat flake Elijah's Manna which would be renamed Post's Toasties and an early version of Grape Nuts which was based on Jackson's Bran pellet. Marketing would continue to point to the benefits of cereal as is seen in the early ads of General Mill's Wheaties which still uses the original "The Breakfast of Champions" 1924 slogan. As competition grew production methods were also thought of as miracles or in the case of Quaker's Puffed Rice, "the eighth wonder of the world.". Cereal production methods for cereal began to develop and continues to develop today. At the root of cereal production, the principles are all very similar. There is a feed material that consists of a grain (or many), a cooking step that aids in transforming or gelatinizing the starch base, a cooling phase, a pressure phase and in some cases a

toasting or flaking step (18). Some examples of production methods are gun-puffed products like Golden Grahams, shredded products like Shredded Wheat and a third very powerful tool, and focus of this work, extrusion.

Extrusion has many different applications. Extrusion is paired with gun-puffed products like General Mill's Cheerios or Mom's Best Honey Nut O's, a combination of extrusion and shredded cereal like Quaker's Life Cereal and much more "simple" extrusion expansion products like Quaker's Cap'n Crunch or General Mill's Kix. Extruder expansion utilizes only the extruder to achieve sensory and mouthfeel attributes that Dr. Jackson could have only dreamed of. Despite being called simple, extrusion is far from that. The extruder has been called a "black box" of production, as it is a system that can simultaneously provide heating, pressure cooking, shear mixing and friction play roles during production. Additionally, these things can be controlled by operators.

Extrusion of Corn Based Products and Flavor Development in the System

While there are many extruded products our focus will be corn based products. A basic diagram of extrusion can be seen Figure 1.2. Many of the principles are the same across ingredients however there are certain challenges associated with certain ingredients. First a brief description of extrusion as applied to corn flour will be presented.

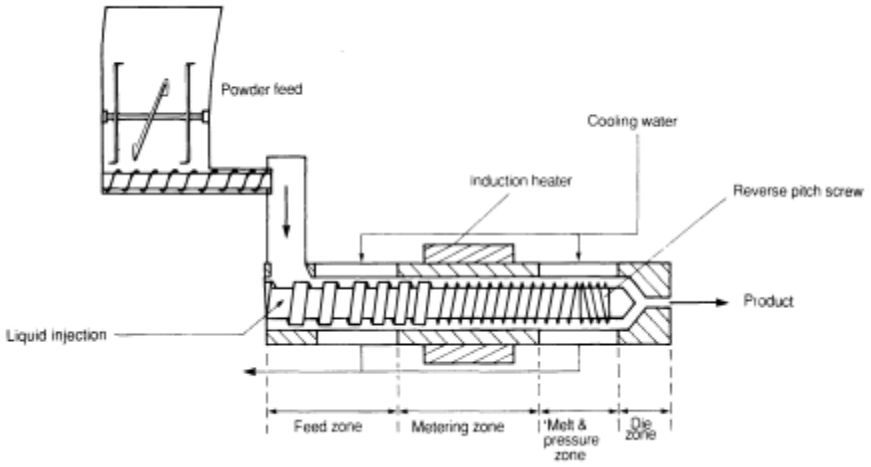


Figure 1.2: Cross Sectional Side View of an Extruder (19)

Extrusion is a thermal processing technique that can mix and pump via screws that are housed in the extruder barrel. To produce corn based foods, corn flour and other dry ingredients are added to the barrel along with water. Once in the barrel the heat and screw action or torque mixes the ingredients into a gelatinous dough. The screw can be designed to put different amounts of work into a food material at different points of the process. An example of where the screw work load is increased is seen in the 'Reverse pitch screw' portion of figure 1.2. The screw design can have different impacts on the product through interactions with the chemical components of the food.

Each component of the dough reacts differently to the heating. Carbohydrates become less viscous while proteins become more viscous when heated (20) and lipids provide lubrication to the system. During this portion of processing, in addition to the steam or oil used to heat the barrel, the mixture is subjected to heat through internal friction of the dough and dough sticking to the wall of the barrel. Ultimately the dough

reaches the end of the barrel where the it is relieved of heat and pressure. The relief from the pressure and heat releases steam and expands the starch matrix which cools to form crunchy textures characteristic of extruded goods. The heating and mixing allows for the development of flavor compounds including non-desirable ones (21,22).

Extrusion processing can play a very important role in the development of flavors (23) via reaction pathways such as non-enzymatic browning, lipid oxidation, and phenolic degradation. The compounds produced by these reaction pathways interplay and create the symphony of aroma and taste that is recognized as flavor.

Flavor research of extruded goods is limited. Existing studies have identified the compounds produced during extrusion and have done so very well, but they have been mainly focused on aroma compounds produced at the die of the extruder (21). Compounds that have been identified in extrusion include many Maillard, lipid oxidation and phenolic degradations compounds (21). However, the studies do not typically aim to understand the compounds that are present in the actual extruded corn puffs or how resulting flavor vary between whole grain and refined ingredients.

Aroma Formation Pathways

Aroma, as a basic building block of flavor perception, can impact consumer acceptance and drive food choices. Thus, understanding the reaction mechanisms responsible for aroma development can affect product optimization. Aroma is generated through several reaction pathways including; lipid oxidation, phenolic degradation pathways, and the ubiquitous Maillard reaction (figure 1.3). Each of these pathways is

produce unique aroma compounds but they can also interplay and contribute to aroma and flavor generation.

Lipid Oxidation

Lipid oxidation pathways are either enzymatic (lipoxygenase action) or chemical (autoxidation). The reaction pathway is influenced by several factors derived from both the production and storage conditions like heat and the chemical characteristics of the food like the lipid fraction.

Lipoxygenase reactions occur when the enzyme cleaves a lipid compound. The cleavage of a lipid compound results in the formation of new compounds that are then free to rearrange or further react, producing aroma volatiles.

The second major type of oxidation is autoxidation. Autoxidation at its core consists of three major parts, initiation, propagation and termination. Due to this, the auto-oxidation can be thought of as a cascade of reactions. Part of this body of work is looking at compounds produced through lipid oxidation reaction as they play a role in understanding the aroma outcomes of food products. The primary aspect of lipid oxidation that will examined is autoxidation as compounds coming from this pathway have been identified previously in extruded corn systems (23,28). Autoxidation can be initiated through several factors like light, temperature and heat. It is common for the factors that influence autoxidation to be controlled during production, storage and product distribution to avoid development of negative flavor attributes.

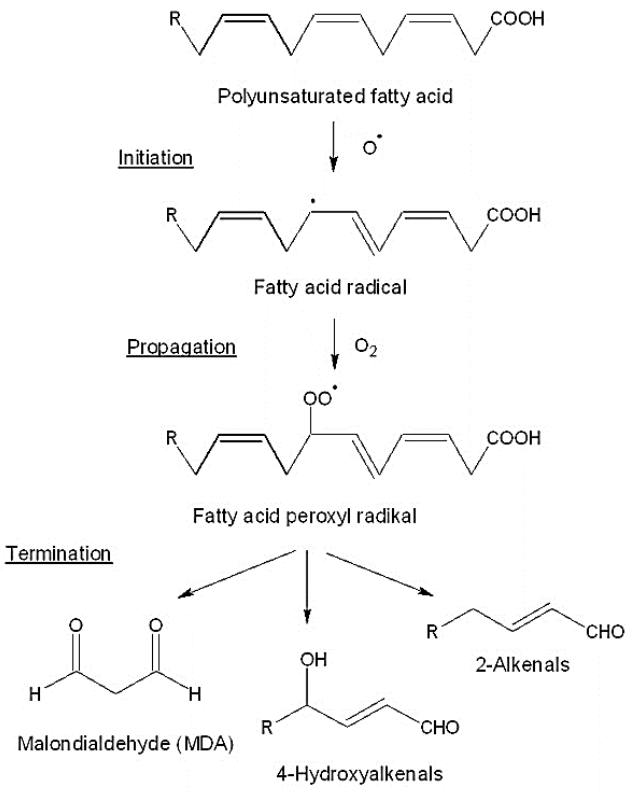


Figure 1.3: Lipid Oxidation Pathway (24)

Oxygen in the atmosphere is typically found in what is called the triplet state or a state that fulfills Hund's rule of filling orbitals with electrons with before they begin to pair. One of the ways that the autoxidation cascade initiates is through the interaction with singlet oxygen (24), +½ and +½ electrons being distributed across the outermost 's' orbitals, this is more energetically favorable. Filling the outer orbitals is where the deference occurs. Singlet oxygen is an 'excited state' that forms through the introduction of energy sometimes in the form of light. In the singlet oxygen electrons of opposite spin occupy the same orbital in +1/2 and -1/2. The resultant singlet oxygen has no unpaired electron and an unoccupied orbital. Oxygen therefore can more easily interact through the

open orbital. Interestingly this interaction has also highlighted the readiness for single oxygen to interact and initiate lipid oxidation as singlet oxygen is observed reacting 1450 times faster than triplet oxygen. One interaction that occurs readily is with unsaturated double bonds of lipids.

Oxygen radicals are another way that the autoxidation cascade can be initiated. Radicals can be formed via an electron loss from diatomic oxygen allowing it to bond with water forming hydrogen peroxide which, then readily breaks down into radical oxygen species. One example lipid oxidation is linoleic acid which is a key fatty acid in corn and other grains. Linoleic acid has been shown to be result in the formation of 2,4-decadienal and 2-pentylfuran which have been identified in extruded corn products in the past (26,23).

Maillard Reaction

The Maillard reaction is ubiquitous in food and changes color, nutritional and flavor. The reaction much like lipid oxidation is a cascade of pathways beginning with the condensation of a reducing sugar with a free amino group (26). The amino group can be free or part of an amino structure like a protein. Following the condensation, the product rearranges into an an Amadori compound. At this point in the reaction pH dictates the next step. If the pH is 7 or below, 1,2-enolisation is the preferred reaction mechanism forming a 3-deoxysone intermediate. Alternatively, if the pH is above 7 the Amadori compound will undergo a 2,3-enolisation forming, 1-deoxysone. As the reaction progresses the Amadori compounds undergo retro-aldol or beta-elimination to generate

C2 to C5 sugar fragments or carbonyls. Sugar fragments are known to propagate the reaction further, generating the color and aroma compounds (27).

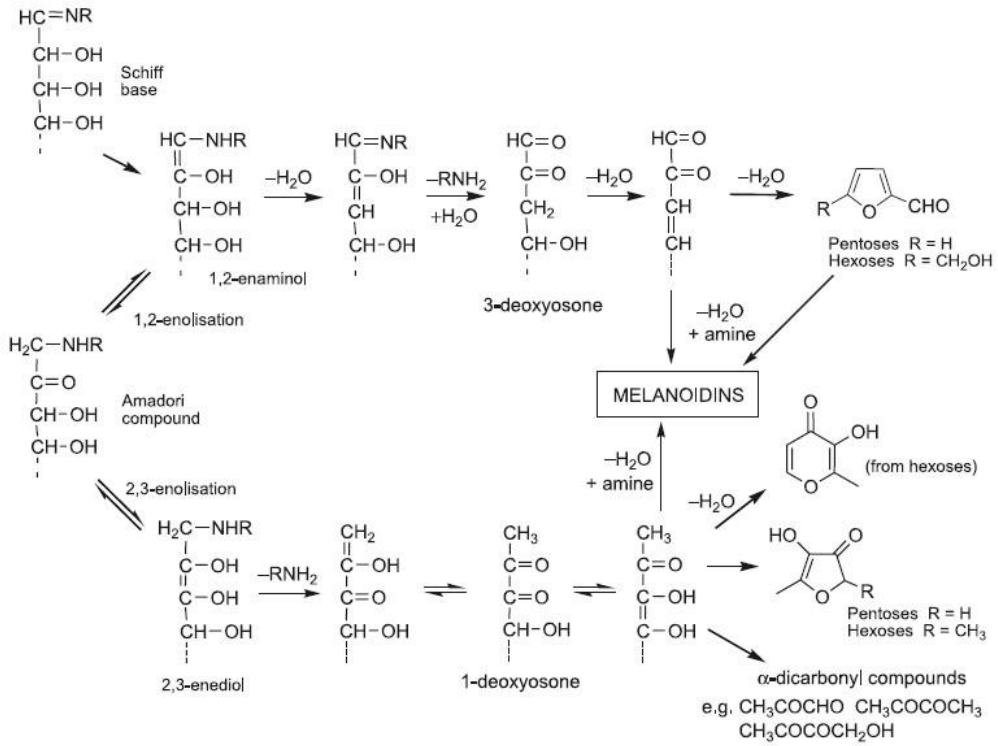


Figure 1.4: Schematic of the Maillard Reaction (27)

Predicting the compounds formed via the Maillard pathways and their impact on aroma can be very challenging, as minute changes in conditions and inputs can result in major changes over the course of propagation. The following represent a few known pathways of formation from given inputs. The first is reactive carbonyl compounds reacting with free amine groups, these reactions will incorporate compounds that have nitrogen in the products. Examples of reaction products from this pathway are in the pyrazines family which can produce aromas like peanut butter or roasted grain a schematic of this reaction can be seen in Figure 1.3. A second reaction mechanism that

incorporates amino acids is the Strecker degradation pathway, the resulting compounds include aminoketones and aldehydes.

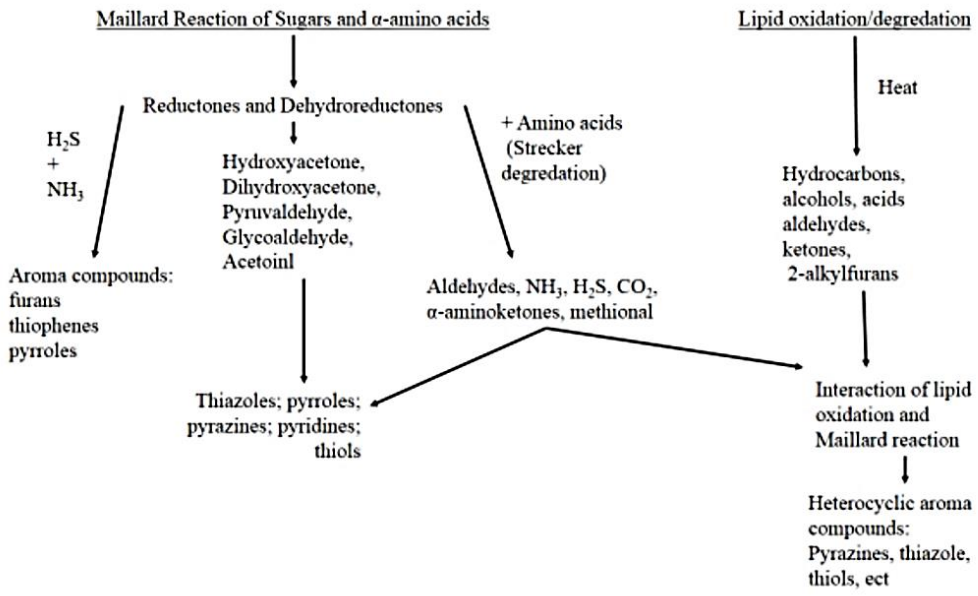


Figure 1.5: Maillard and Lipid Oxidation Reaction Pathways and Interaction (28)

The Maillard reaction's influence is not limited to the formation of only aroma compounds. As mentioned above the Maillard reaction produces many compounds. One example that provides a bridge from aroma to taste is the formation of bitter compounds in low moisture systems, like roasting (39,25).

Phenolic Degradation Compounds

Phenolic degradation compounds can arise from compounds including organic acids and polysaccharides. Hydroxycinnamic acids or HCAs are organic acids that are found in free form or in association with other components of plants (29,32).

During processing, HCAs can undergo degradation reactions like decarboxylation and oxidation that result in the formation of aroma compounds. One notable HCA is ferulic acid. Ferulic acid has been associated closely with the formation of aroma compounds such as guaiacol, 4-vinylguaiacol and vanillin. Phenolic compounds contribute to the aroma of sweet corn (32), popcorn (33) and tortillas (34). Elucidating the mechanistic pathways of ferulic acid degradation is important as it occurs in a variety of products and affects the overall flavor profile.

As seen in figure 1.4 ferulic acid undergoes a decarboxylation to first form 4-vinylguaiacol followed by an oxidation reaction to form vanillin followed by a second oxidation that yields vanillic acids and a final decarboxylation that produces guaiacol. 4-vinylguaiacol, vanillin and guaiacol. These compounds have clove like, vanilla like, and a smoke like aromas respectively.

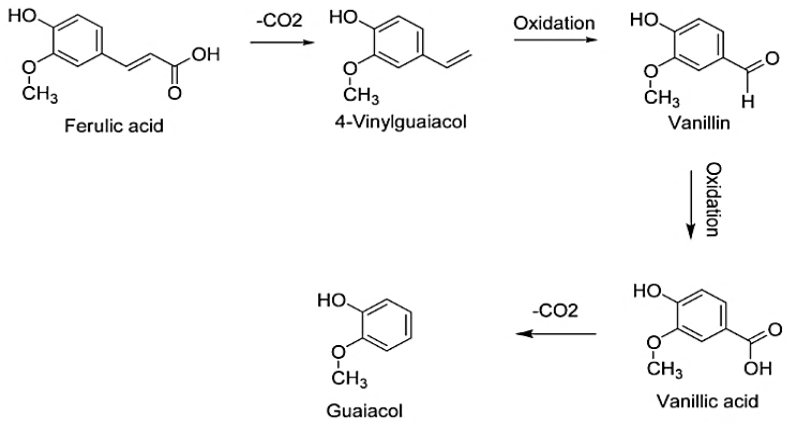


Figure 1.6: Proposed Degradation Pathway of Ferulic Acid into Aroma Active Compounds (35)

HCAs are of interest in aroma analysis and sensory sciences as they play an important role in consumer acceptance since inhibit the Maillard reaction forming compounds (68). Foods with lower HCAs like refined grain bread content having higher consumer liking (36). Interestingly HCAs additionally contribute to taste as they have been linked to bitterness generation.

Taste

Taste can be divided into five major attributes including sweet, sour, bitter, salty and most recently umami. The five major taste attributes are detected through unique mechanisms. Sweet, bitter, and umami are detected through proteins receptors, specifically G-protein-coupled receptors while salty and sour, are detected through ion channels. For the focus of this thesis bitter receptors and detection will be the only taste receptor discussed in detail.

Taste compounds like aroma compounds are soluble, however unlike many aroma compounds they are soluble or semi-soluble in water and saliva, an example of a bitter compound is caffeine. During mastication compounds are spread throughout the oral cavity and gain access to the bitter receptors through the apical proteins. The proteins have specificity in binding, like how enzymes function in other areas of the body. Once the proteins bind to taste active compounds the receptors send a recognition signal to the brain (37). Interestingly, compounds that are part of the same class or category can differ greatly in how they are sensed by the receptors due to differences in hydrophilicity, structure, and size. A prime example of how compounds can differ, even when very

similar, is D-tryptophan and L-tryptophan, pictured below in Figure 1.3. D-tryptophan is sweet while the latter is bitter (37,38). Taste receptors can be thought of generally as chemical sensors.

While taste receptors are important to overall taste experience, saliva plays an important role as well. Saliva secretion is the first physiological behavior involved in ingestion of food. Saliva contains a protein fraction that consists overwhelmingly, about 70%, of the amino acids; proline, glycine, and glutamic acid. Proteins in saliva have been reported as binding agents to taste compounds that are hydrophobic. The binding action is an important one as it enables the diffusion of taste compounds to the taste receptors or taste buds throughout the mouth.

Bitter

The perception of bitterness is complex. Unlike sweetness and umami, which have one type of receptor T1R2+T1R3 and T1R1+T1R3 respectively, bitterness has 30 T2R receptors. The T2R receptors that bitterness is detected by are heterodimeric or consist of two similar molecules, in this case proteins (37,38,39). The heterodimeric characteristic allows for a broad range or multiplicity in binding, allowing for many different compounds to bind the same receptor and be sensed.

Bitter compounds can be seen below in Figure 1.7. Compounds presented in Figure 1.5 are mainly phenolic compounds. Phenolic compounds are very common in plants and typically are bitter. Naringin is found in grapefruit, quercetin is found in wine, and catechin is found in green tea (43). While phenolic compounds are typically bitter

how they are perceived varies based on a few different chemical characteristics, functional group, sugar moiety composition, and stereochemistry.

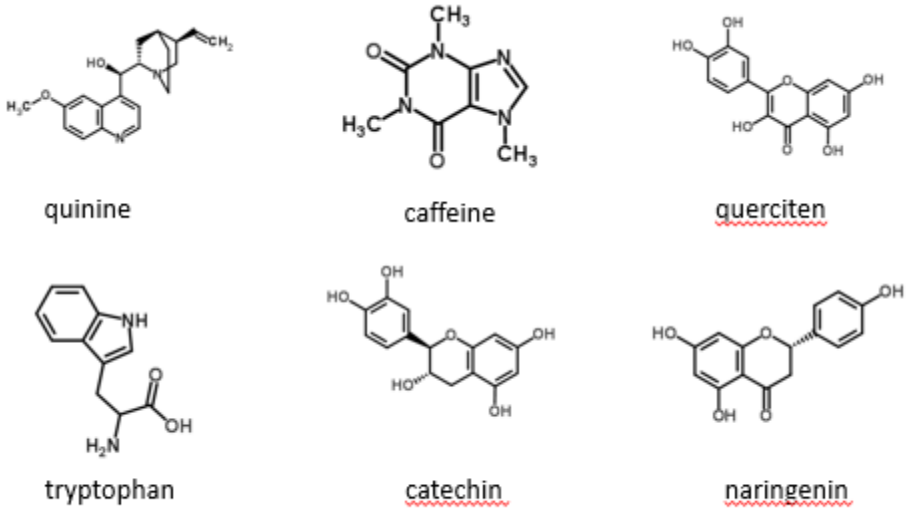


Figure 1.7: Bitter Compounds Found in Assorted Foods (44)

1. Functional groups (2): compounds containing three nitro groups will typically be bitter. Identifying a functional groups' influence the bitterness of a compound was first done in 1895, with the discovery that compounds containing the group found in Figure 1.6, are bitter. In 1963 it was noted by Pearson that bitter tastes are common in compounds that are 'soft' bases. Soft bases refer to the weak adsorption of the compound to the amino acids in the proteins that make up the receptor. In this description of bitterness, the softer the base the more bitterness the compound elicited. In 1984 Saroli suggested that bitter compounds typically consist of two parts; a hydrophilic group and an electrophilic group or base (44). While these finding cast light on to the nature of bitter compounds, functional groups are not the whole story of bitterness. To highlight that functional groups are only part of the picture a database of 500 compounds was published in 2012 by Wiener, none of the

studies used to compile the database suggested the requirement for certain structural characteristics (45).

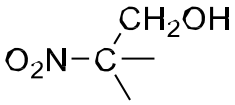


Figure 1.8: Structural Aspect of the First Bitter Compound Identified by Saroli in 1895 (46)

2. Sugar Moiety: Bitterness, in part, on the attached sugar moiety (47). Neohesperidosideses are common in fruits such as grapes and other citrus. The compounds consist of a polyphenolic group attached to an alpha linked disaccharide glyosidic group. When naringin is attached in the neohesperidosidase moiety it is bitter while in other glucosides it is not.
3. Stereochemistry: A common example of how stereochemistry is an important factor in the bitterness of a compound is in sweet D-amino acids and bitter L-amino acids (41). While this is a concrete example for small compounds on larger compounds the stereo chemistry of functional groups may play a role in the bitterness threshold. More study is needed in this area.

Bitterness like other aspects of the sensory system is likely to have played a role in evolution. One potential role bitter sensation is thought to have played is the detection of harmful or poisonous materials. Today many people consume a broader diet when compared to early ancestors with a large emphasis placed on healthy foods that provide

more tailored nutrients based on modern understanding of human nutritional needs. There is also a growing market for what are deemed functional foods that offer the consumer some sort of benefit like a probiotic or prebiotic. While these foods gain popularity, they are also limited with sensory challenges, one of which is bitterness. Overcoming flavor challenges such as bitterness can facilitate wider consumer acceptance of healthy food options and provide great benefit to public health and disease prevention.

Aroma Analysis

Aroma analysis is complex and challenging. Over 1000 unique aroma compounds have been identified with each having unique chemical and organoleptic characteristics. To identify these compounds a wide range of techniques are required beginning with extraction of volatile compounds and traditionally ending with analysis using gas chromatography (GC) and mass spectrometry (MS).

Solvent Extraction

A wide variety of techniques can be used for the extraction and commonly, solvent extraction is viewed as one of the most robust and reproducible methods available for aroma isolation. While there are other methods available for this work like solid phase micro extraction, static and dynamic headspace analysis as well as purge and trap methods, solvent extraction was used in this study to obtain a comprehensive volatile fingerprint and will thus be discussed in the following section.

Solvent extraction relies on the partition of volatile compounds from the food system into an organic solvent. The solvent chosen is based on the compounds anticipated to be present in the food or the analyses of interest.

One of the great advantages of solvent extraction is the simplicity of the methods and equipment required for the initial extraction. Effective solvent extractions can be carried out using separatory funnels, orbital shake tables and centrifugation allowing for the development of cost effective methods.

While there is no requirement for specialized instrumentation during the initial steps of solvent extraction, the technique does have limitations that necessitate the use of specialized equipment to achieve concentration and clean up prior to analysis. Solvent extraction leads to a very broad extraction of volatile compounds and other materials such as proteins and lipids which can complicate analysis. In addition, solvents like all chemicals have their own characteristics which can directly impact compound extraction efficiency based on their affinity to the solvent. Historically non-polar solvents like hexane, di-ethyl ether and methylene chloride have been used for extraction of aroma compounds (48).

Given the non-polar nature of solvents used, issues can arise with the extraction of non-volatile constituents such as fat, which with a few exceptions is found in all food systems. The extraction of fat is of concern as it can affect the chromatographic separation and cause undue wear and tear on analytical instruments, such as decreased column life and fouling of sample inlets. Both of which can lead to poor chromatography and/or issues with compound identification.

To overcome these challenges “clean-up” methods, for further purification of the volatile fraction, are employed. A current method used for sample cleanup is Solvent Assisted Flavor Evaporation or SAFE.

SAFE is highly effective method across a wide range of food matrices including systems with high percentages of fat (49). SAFE allows the removal of non-volatile components and the isolation of even trace volatile compounds without artifact formation or degradation. SAFE utilizes a high vacuum and physical barriers to ensure collection of the volatile fraction free of non-volatiles. As seen in Figure 1.7 there are two round bottoms flasks attached to the system. The sample is introduced to the system via a stop cock into the first round-bottom flask (left). As the sample is introduced into the system it is exposed to the vacuum. As extract is introduced to the first round-bottom flask the solvent and volatile compounds are evaporating and move through the center of the SAFE into the second round-bottom flask (right) or volatile collection flask which is kept in liquid nitrogen. The center of the SAFE incorporates physical barriers that prevent non-volatiles such as fat and protein to be transferred to the volatile collection flask.

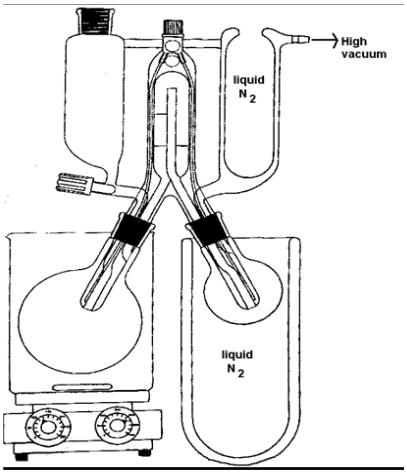


Figure 1.9: Schematic of SAFE Apparatus

While all of this is done effectively and with limited negative impact on the sample, SAFE does have drawbacks. One of the major challenges is the time that is required for the SAFE system to be set up and the volatile isolation to be completed as well as the cost associated with the specialized glassware and high vacuum pump necessary to run the system. Additionally, the reproducibility of the system could suffer when vacuum changes occur and results can be highly dependent on user and sample introduction.

GC, GC-O and GC-MS

Gas Chromatography (GC) is a widely used analytical tool. GC is a separation technique that allows operators to separate a mixture of compounds into individual compounds. Separation is achieved through two means. The first way compounds are separated is by boiling point. This is achieved through a heating profile, sometimes referred to as the temperature ramp.

A basic ramp increases linearly over the course of a chromatographic run. As the ramp increases compounds begin to volatilize and travel through the GC column. Compounds may have very similar boiling points but have very different chemical properties. The second mode of separation occurs in the column, which exploits chemical differences. Most separations occur based on polarity, however specialized columns can be designed to separate based on other characteristics such as chirality. For this work polarity was the characteristic that separation was based on.

Inside the GC column there is a phase of a specific make up, that the compounds travel above as they move through the column. As the compounds move through the column if they are polar and traveling through a polar column they will have an affinity to phase. The affinity will result in an interaction that slows the movement of the compound through the column and thus will affect their retention time on the column. An analogy for a chromatographic separation is a moving walk way. If a person stands on a moving walkway they will be moving but at a slower rate than a person walking would be. Upon reaching the end of the column the compounds emerge and travel through a detector, like a mass spectrometer (MS) or in the case of Gas Chromatography-Olfactometry (GC-O) to an operator.

GC-O is a specialized technique that has wide spread use in the flavor and fragrance industry. What makes GC-O unique is that it links a human sense to GC separation capabilities (51). GC is a separation technique that has an enormous ability to separates volatile compounds. All volatile compounds are odor active. GC-O bridges this gap. As the compounds emerge from the end of the column a split can carry compounds to a detector as will be discussed later or down a heated arm with a transfer line leading to an olfaction port. At the olfaction port an operator can report the odor descriptors of the compounds eluting off the column. This is where the differentiation between aroma active and non-aromatic volatile compounds can be observed.

The observation allows the operator to report the aroma and the retention time of a compound. Both characteristics are used in subsequent identification steps. Compounds that are aroma active and volatilized by GC haveunique aroma to the operator.

Additionally, the operator can use the detection time to note the Linear Retention Index (LRI).

The LRI is also referred to as the Kovat's Index. The LRI of a compound is calculated based on the retention time and the relationship it has with respect to n-alkanes during a linear ramp (Figure 1.8) In a linear ramp n-alkanes will elute at regular intervals. Each window is labelled by the n in the previous alkane. Thus if a compound elutes following nonane it will have an LRI of 900 to 999 and a compound eluting following decane will have an LRI of 1000 to 1099 and so on. Once the descriptor and LRI have been determined the compounds can be compared to databases of aroma compounds like FlavorNet (50).

$$I = 100 \times \left[n + \frac{t_{r(\text{unknown})} - t_{r(n)}}{t_{r(N)} - t_{r(n)}} \right]$$

Figure 1.10: Kovat's Index Calculation

In addition to the preliminarily description and LRI assignment to an aroma extraction dilution analysis (AEDA) can be used to gain qualitative insight into concentration differences between samples (52). AEDA is a simple method; the extraction is diluted by a given factor in a serial fashion. Each dilution is analyzed through GC-O and as compounds reach their aroma active threshold they will no longer be observed during

GC-O. AEDA is commonly thought that the compounds that are present in subsequent or more dilute samples are the most important for the aroma characteristics of a product. Understanding which compounds are most important can allow for the comparison of two foods (53). AEDA was used as a comparison tool to guide analysis. For the purposes of this work AEDA was used to describe and identify the aroma active differences between whole grain flour and refined corn flour puffs. AEDA can be applied independently to two ingredient foods. Once the two profiles have been developed they can be compared. The comparison can show unique compounds and qualitative concentration differences through the AEDA dilution factors. Once this step has been carried out the differences can be identified and verified through further analysis like mass spectroscopy.

Mass Spectroscopy

To determine the identity of a compound a mass specific or mass spectrometer detector (MS) is employed. A MS can be thought of as a highly-sophisticated scale, that can measure the mass of ions and their fragments. To make the measurement the compounds emerging from the GC column must first go through a series of steps. The basic steps include ionization, ion separation and detection (54).

The first step, ionization occurs directly after the column as the ion source or simply the source interacts with the molecule exiting the column. While there are multiple types of ionization chemical ionization (CI) and electron ionization (EI) will be discussed.

In a CI source the analyte is protonated with a reagent gas. In the CI source the analyte is introduced to an ion beam along with the reagent gas. The analyte and the reagent gas ionize and react with one another. The result is the reagent gas to be effectively added to the analyte. In this type of source the resulting compound does not fragment and is regarded as a soft ionization. This is a technique that is particularly useful for the detection of compounds that might not be observable in hard ionization techniques like EI.

In a EI source a filament is positioned between a positively charged repeller plate and a negatively charged accelerator plate, each with a hole in them to allow for compounds of ions to travel through. The filament emits a high-energy electron beam that strips electrons from the compound causing the formation of cations. In GC-MS applications the energy of the electron stream is 70-Ev, this is a standard setting and allows for comparison across MS data bases. The repeller plate pushes the cations forward through the accelerator plates, some systems have multiples. Following the accelerator plates there is a negatively charged focusing plate that focuses the cation beam directing it to the magnetic field.

The magnetic field is part of the mass analyzer. In the mass analyzer, the ions are separated by mass-to-charge (*m/z*) ratio. There are a few types of mass analyzers available, for this work two were used a quadrupole and a time of flight.

Sensory Analysis

After compound identification, their influence on the overall flavor profile of foods an example of sensory analysis like was demonstrated by Potts in 2016 (54). There are several sensory testing methods available including, flavor profiling method, quantitative descriptive analysis and quantitative flavor profiling but for this work descriptive analysis (DA) test will be discussed (54).

The DA test relies on panelists to screen and rate the intensity of an aroma compound. The panelists are selected based on their ability to recognize aroma attributes and provide intensity ratings. Once the panelists are selected they are further trained to familiarize with the lexicon of aroma descriptors in the study, of the study. This is done to standardize the descriptors that will be used and is performed using standard compounds or foods that embody the odor descriptor. When the panelists are comfortable with the lexicon testing is carried out. Testing is performed by comparing the sample with the identified compounds in a recombination model. Recombination models consist of a model food matrix like starch that has compounds of interest added to it. The sample's identity is unknown to the panelists throughout the test. The intensity of the descriptors in the samples is rated on a 15-point linear scale. Upon completion of the test the data is statistically analyzed.

Bitter Compound Identification

The use of GC-O allows the analyst to determine which compounds are aroma active by separating them on a GC column and to characterize their sensory attributes by smelling them through an olfactory port. In identifying taste active compounds, analysis

is carried out concurrently with tasting. The concurrent fashion of the analysis leads to what is called sensory guided analysis. In other words, researchers are narrowing the scope of analysis based on feedback from a taste panel. Sensory guided fractionation requires the use of preparative or semi-preparative high pressure liquid chromatography (HPLC) coupled with fractionation techniques. An extract derived from a food matrix is injected onto the HPLC, chromatographic separation occurs on a LC column and fractions of analytes are collected as they elute from the column. After solvent evaporation and dilution in water fractions are evaluated by panelists for the presence of the attribute of interest.

Due to the high complexity of food matrices and their extracts, often fractions or chromatographic peaks can contain more than one co-eluting compounds. To identify the taste active compound in a fraction, subsequent fractionation is carried out on a chromatographic column with an orthogonal chemistry. When sufficient purification is achieved, further characterization is performed via accurate mass time of flight (ToF) analysis and NMR for structure elucidation.

Taste dilution analysis (TDA) and taste activity (TAV) and DoT concepts

Like to established concepts in volatile research, such as aroma activity, and methods for dilution analysis, the taste activity value was recently defined (57). Taste dilution analysis (TDA) has been developed as a screening procedure for taste-active non-volatiles in foods (56) and has been used in multiple studies since (59, 61, 62). The typical procedure includes serial two-fold dilutions of HPLC fractions,

with water and evaluation of those fractions using a sensory panel and the half-tongue test in ascending (concentration) orders. The dilution at which a taste difference between the diluted extract and the blank (control) could just be detected is defined as the taste dilution (TD) factor. Higher TD values are believed to correlate with greater taste impact (57). An example of TDA analysis is presented in figure 1.11.

TDA represents a systematic method that requires reduced panelist training, and is more numerically reproducible in screening for taste compounds with low attribute thresholds. However, taste perception and intensity of attributes is not linearly correlated with concentration of the compound. For example, compound A may have a higher TD value (lower bitterness threshold) when compared to compound B while also having a lower bitterness intensity than compound B when both were at higher concentrations.

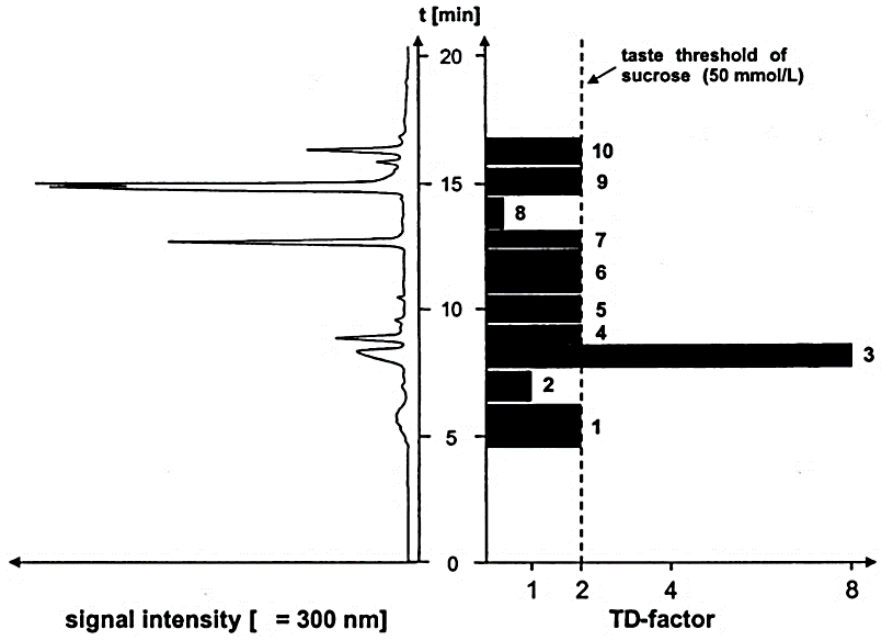


Figure 1.11: Taste dilution analysis of screening taste-active fractions from HPLC separation (51)

In analogy to the aroma activity value, the taste activity value (TAV) is defined as the ratio between the concentration and the attribute (e.g. bitter, sweet, umami, etc.) threshold concentration of a compound in food, which can be used to evaluate the contribution of a compound to the overall attribute (57). Dose-over-threshold (DoT) is another effective method for identifying the important taste contributors in a given food. The approach relies on rating the intensity of the attribute compared to known intensity reference standards. The compound is compared to the known intensity standards at the concentration that it is found in food systems providing insight regarding the impact of a compound to the attribute of interest (57-59).

The above-mentioned approaches only study individual compounds, and puts those compounds out of the context of food matrix. When a taste compound is present in food, many factors could contribute to its perception, such as its solubility in saliva. The forces that govern the dissolution of taste compound in saliva during food mastication are complex, and some taste compound are more likely to be extracted by saliva and to be perceived than others.

Modulation and Mitigation of Negative Flavor Attributes

Understanding how the flavor profile of a product is impacted by processing and ingredients can lead to the development of control points and optimization methods that provide better consumer experience. Flavor quality improvement can be achieved through a variety and the approach can change with the product at hand. Different processing techniques like pre-processing heat treatments (68) have been used or more

targeted solutions such as the addition or removal of individual ingredients (69, 70). Development of off flavor formation mitigation strategies requires a line of investigation that builds upon carefully acquired prior knowledge and begins with observation.

How simple processing techniques can impact the flavor development in products was observed by Usol (67). A mild heat treatment of 50 °C was applied to whole melon prior to cutting cantaloupe melon. The melons were then stored for eight days at 10 °C. Following the storage, the heated stored melon was compared to non-heated stored melon by a panel of 11 trained sensory testers. The heated group showed had a decrease in negative sensory attributes including metallic and bitter attributes. This was noted as potentially being the result of a lower enzymatic activity following the heat treatment. This study highlights that in efforts to improve flavor there might be simple solutions producers can use. However, the authors did note that this study was merely a stepping stone and understanding more complex chemistry of what is occurring begs further more targeted investigation.

An example of how a deep understanding of chemical interactions can result in a better understanding of flavor and improved attributes is shown in Moskowitz (69). In the study conducted Moskowitz (69) the impact of hydroxycinnamic acids (HCAs) on aroma characteristics in whole grain and refined bread was examined. HCAs are naturally occurring in grains and are quantitatively negligible in refined grain compared to whole grain as they are found in the outer portions of the grain such as bran and hull that are removed during milling.

Following a GC-O guided examination of the aroma development pathways in both whole grain and refined bread potential pathways were examined. During the examination of the aroma development pathways ferulic acid was identified as playing a role in the suppression of aroma development in whole grain bread. The study then examined the impact of ferulic acid through addition of the compound to refined bread, to determine if it could suppress the formation of a vital aroma compounds 2-acetylpyrolline (2-AP). When ferulic acid was added into the refined grain system at a concentration consistent with whole grain flour five key aroma compounds, including 2-AP, formed in lower amounts. Ferulic acid was found to be higher in the whole grain bread following production and this is likely due to continued liberation of the acid. This study demonstrates the importance and power of natural ingredients on the final product flavor quality. The study highlights the importance of identifying high impact compounds in flavor pathways which can lead to the development of processing methods or ingredient technologies that improve flavor perception.

In a study conducted by Kokkinidou (70), the impact of phenolic compounds on the Maillard reaction pathways and the impact on the cooked off-flavor attribute of UHT milk was explored. an ingredient addition approach was developed and its effectiveness in inhibiting Maillard reaction pathways was examined. The approach employed the addition of polycyclic phenolic compounds, derived from varied food sources, prior to UHT processing of milk. In the UHT system phenols limited the formation of compounds formed during the Maillard reaction responsible for an unpleasant burnt or cooked aroma. The inhibition mechanism proposed was a formation of a phenolic adduct with reactive

carbonyl species. The study demonstrated how addition of natural phenolic compounds can be used to effectively trap and remove reactive species in a food system resulting in reduced off-flavor formation.

Off-flavor mitigation methods can incorporate simple pre-processing steps or addition of natural ingredients but no matter what the approach is a deep understanding of ingredient (whether native or added) interactions and associated flavor formation pathways is necessary.

Bitterness Mitigation in Extruded Corn Puffs

In 2013 Zhang and Peterson identified chaenorpine in whole grain extruded puffs for the first time and sensory revealed its high impact of bitterness perception in the product (67). Given the relatively limited available information for chaenorpine in food systems, the impact of food processing methods, such as extrusion is not known. Thus, hindering the development of practical solutions for its mitigation and the reduction of bitterness in whole grain products.

To limit the amount in the final product how the compound is impacted during processing or how certain changes in processes impact the compound during production needs to be defined. Many factors that can impact the sensory characteristics of a given food including ingredient handling and processing. These two factors are easily controlled by food and can have a great impact.

Ingredient handling can have a profound impact on final product quality and characteristics. There are many variables that can impact an ingredient like flour during

storage, including heat, time, moisture, light, and packaging are factors that can impact product quality. The impact of storage on corn flour used to produce roti, a flat bread common in India produced at high temperatures over a short time of approximately 60 seconds was studied. Flour that was stored for six months in poly ethylene showed an increase in bitterness and marked decrease in overall acceptability (72). The flour was noted to have different characteristics from the fresh flour including decreased water absorption capacity, decreased oil absorption capacity and a decreased pH. This indicates that storage plays a role in the characteristics of flour and potentially the how the flour behaves during processing.

Two factors changed during storage in the Shoba study (73), that are important to note are the water absorption capacity and pH. These two factors are important for how the Maillard reaction progresses as a higher pH and a lower water content lead to accelerated rates of the reaction. The Maillard reaction is typically known as a reaction that is vital for the formation of aroma in food systems however compounds formed in the Maillard are markers for bitterness (69).

In 2012 Bin (74) demonstrated the correlation of Maillard browning compounds and increased bitterness in whole grains. In the study four commercially available 100% whole grain wheat breads and one refined grain wheat breads were analyzed for bitterness. The study quantified eight previously identified bitter compounds present in whole wheat bread to characterize the impact of each compound on the bitterness perception of whole wheat bread (64). Results revealed highly significant correlations ($\alpha = 0.01$) between the perceived bitterness in the crust and the quantity of 5-

(hydroxymethyl)furfural ($r^2 = 0.93$) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one ($r^2 = 0.95$). In the crumb, the bitterness was correlated with the amount of l-tryptophan ($r^2 = 0.91$). In summary, both Maillard- and fermentation-derived compounds were identified as potential chemical markers to predict bitterness of whole-wheat bread (71). The Maillard reaction was also noted as being responsible for many compounds observed in extruded corn puffs (21, 22). By altering the conditions like pH and water content to promote or inhibit the Maillard reaction it might be possible to influence the overall bitterness of the extruded corn puffs.

Research Objectives

The overall aim of this work was to determine impact of food composition and processing on flavor generation of whole grain products, to facilitate the development of flavor optimization strategies for healthier food choices.

Increased consumer awareness regarding the impact of diet on health and disease prevention creates expectations for healthy and tasty food products in the market. The US Dietary Guidelines recommend half of all grains consumed be whole. Whole grain products suffer from low consumer acceptability. Negative aroma attributes as well as bitterness are known to adversely impact the flavor quality and the consumption of whole grain foods. One step in understanding and developing solutions for mitigation of negative sensory characteristics is understanding the compounds that drive the perception.

As consumers look to expand their consumption of whole grains, ready-to-eat cereal provide a great platform due to convenience, availability and market penetration. Thus, this work attempt to further understand flavor formation mechanisms in whole

grain extruded corn puffs. The aroma differences between whole grain and the refined corn puffs are discussed in chapter 2 while the impact of processing conditions and ingredient aids on the content of a key bitter compound, chaenorpine, is discussed in chapter 3.

The major research objectives of this study are:

1. Identify and measure the aroma differences between whole grain and refined extruded corn puffs
    a. identify key aroma compounds and mechanistic pathways of generation
2. Examine the impact of processing parameters on the concentration of key bitter compound, chaenorpine in extruded whole grain corn puffs

Chapter 2: Characterization of Aroma Differences in Refined and Whole Grain Extruded Corn Puffs

SYNOPSIS

The influence of corn flour type (refined versus whole grain) on the aroma generation of extruded corn puffs was investigated. Aroma profiles were monitor via GC-O-MS and differences were characterized by aroma extract dilution analysis. For whole grain corn puffs (WGP) 13 compounds were observed as contributing to the aroma differences. With 12 being higher in concentration in the WGP sample including hexanal, 2-methyl-pyrazine, 2,3-dimethylpyrazine, 2-pentylfuran, 2-ethyl-3,5-dimethylpyrazine, 3-hydroxy-2-methyl-4H-pyran-4-one, 2-methoxyphenol, 2-acetyl-2-thiazoline, 2,4-decadienal, 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde whereas in refined grain puffs (RFP) 2,5-dimethylpyrazine was found to be higher in the RFP sample. All compounds with the exception of hexanal, 2-pentylfuran, 2,4-decadienal, methythiazoline and 2-methoxyphenol were found to have significant concentration differences. Descriptive analysis was carried out using six aroma descriptors: cooked, corn chip, oxidized, roasted, sweet vanilla and toasted. Descriptive analysis results further supported the analytical data, a higher mean aroma intensity value was found for cooked, corn chip, roasted, toasted for WGP samples compared RFP samples. In summary, the differences in aroma perception between WGP and RFP puffs is influenced changes in the concentration of Maillard reaction products.

INTRODUCTION

Consumption of whole grain corn has been associated with a variety of health benefits including body-weight regulation, reduced risk of chronic pathological condition, reduced blood glucose levels (97, 100, 108). However, less than 5% of Americans consume the recommended intake (48 g/day) and up to 20% consume no whole grain products (113). Increasing consumption through familiar products could help expand these health benefits.

Ready-to-eat (RTE) whole grain breakfast cereal is a product platform with an established consumer base (103). Due to the consumer base and market share RTE cereal provides a great avenue for consumption of whole grains. Alas, food choices are mainly driven by flavor and the negative attributes associated with whole grains have been reported as one of the most influential factors limiting consumption (77). This is especially true for children which (110), per a recent study on cereal consumption, are key driving force of purchasing (103).

To accommodate the flavor preference of children and many adults, many commercially available whole grain products and especially cereals contain added sugar, salt and flavors to mask negative attributes in the flavor profile. Therefore, to increase the consumption of whole grain cereals while maintaining a smaller ingredient label in line with today's consumer demands, it is important to improve the palatability by understanding how compounds endogenous to the grains drive negative flavor attributes.

Previous work has been carried out to understanding the impact of thermal processing and more specifically extrusion on aroma generation (102). The impact of specific extrusion cooking conditions like heat, water content, and residence time have been shown to exert significant effects on the flavor profiles of food products (115) and

the cooking temperature was shown to influence the formation of flavor compounds (92). While studies have been carried out investigating flavor development in corn whole grain extruded corn products (79,102) many are dated and there is a lack of direct comparison between products formulated with whole grain versus refined corn flour.

Understanding how the compositional differences between whole grain and refined flour affect flavor development can provide insight regarding the mechanistic pathways responsible. The objective of the current study was to investigate the influence of corn flour type (whole grain and refined) on aroma development of extruded corn puffs, identify the key chemical differences and their respective sensory attributes and provide further insight regarding the chemical pathways involved.

MATERIALS AND METHODS

Twin Screw Extrusion

Extrusion conditions were designed to yield uniform cell structure throughout each puff (79). Briefly, extrusion processing was carried out using at The Joseph J. Wartheson Pilot plant with a Buhler DNDL-44 twin screw extruder, two formulations were used: 1. a refined corn flour formulation and 2. a whole grain corn flour formulation. The refined corn flour dry formulation consisted of 970 g (97%[DP1]) refined corn flour (Innovasure™, Cargill MN) with 10 g (1%) trisodium phosphate, 10 g (1%) calcium carbonate and 10 g (1%) sodium chloride. The whole grain corn formulation consisted of 465 g (48%) (Innovasure™, Cargill MN) refined corn flour and 505 g (52%) whole grain corn flour (Maizewise™, Cargill, MN) with 10 g (1%) tri-sodium phosphate

(TSP), 10 g (1%) calcium carbonate and 10 g (1%) sodium chloride. The ingredients were added to a mixer and mixed for 10 min. The mixture was added with 14% (w/w) water into the extruder with a low work screw configuration via feeder and processed per the following extrusion parameters: computer controlled shaft speed of 350 rpm, a measured die pressure of 10.1 ± 0.5 bar, die temperature of 160 ± 1 ˚C, material throughput of 50.8 ± 0.1 kg/hr. with 7 kg/hr. water and a cutter speed of 1200 rpm resulting in puffs. Due to differences in the physical and chemical characteristics of the refined and the whole grain flour mixes, the refined corn flour formulation showed an increased shaft torque of 224 N M over the whole grain corn flour formulation of which had a shaft torque of 215 N M; the specific mechanical energy for refined corn flour formulation was 164 kw/h while the whole grain corn flour was 159 kw/h. Other parameters were constant across both formulations. The puffed product was collected, dried on a liquid air bed, and stored in high density polyethylene bags at -40°C for later analysis.

Solvent Extraction

To achieve a comprehensive aroma extraction a multi-solvent protocol was developed. Briefly, 300 g of corn puffs were ground and placed in 1-liter Erlenmeyer flask. Next, 600 g of GC-Resolv® methanol (MeOH) (Fischer Scientific, Pittsburgh, PA) spiked with 0.1 ppm 4-heptanone, (Sigma Aldrich Milwaukee, WI), was added to the flask which was then shaken for 24 hours on a orbital shake table set at 200 rpm. Methanol was collected and the ground corn puffs were re-extracted for 2-hours using 400 g of methanol, at 200 rpm. Organic layers were pooled and 600 g of the methanol

collected was subsequently combined with 600mL of reverse osmosis purified water. The water-methanol mixture was then poured into three 1-liter separatory funnels and extracted using 500 g of GC-Resolv® methylene chloride (DCM) (Fischer) spiked with 0.1 ppm 2-methyl-3-heptanone, (Sigma Aldrich Milwaukee, WI). DCM was added in 100 mL aliquots to each funnel for a total of 5 extractions. The methylene chloride extract was then placed in a -20˚ C freezer overnight to separate and remove any residual water-methanol. The DCM extract was collected and then dried using sodium sulfate and subsequently concentrated via distillation to 1.0 g. The concentrated extract was stored at -80˚C until analysis. A flow chart of the extraction protocol is displayed in Figure 1. Additionally, the internal standards used were analyzed for reproducibility during extraction, MeOH was spiked with 4-heptanone and DCM was spiked with 2-methyl-3-heptanone which were found to have a variance of 4% and 3% respectively (n = 3). This method was found to be have a higher number of aroma active regions in GC-O analysis compared to a single solvent DCM extraction coupled with Solvent Assisted Flavor Extraction (SAFE) as outlined in previous work (101). Comparison was performed via GC-O and on average the methanol extraction protocol reported 111 aroma active regions, whereas methylene chloride extract protocol reported 86.

Gas Chromatography-Olfactometry-Mass Spectrometry (GC-O-MS): Aroma Extraction Dilution Analysis (AEDA)

GC-O analysis was performed on a GC (Hewlett-Packard, 6890, Santa Clara, CA, USA) equipped with a DB-5 column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) coupled with a MS (Agilent 5973, Santa Clara, CA, USA) operated in EI mode. the system was also equipped with an

olfactometry port (Gerstel, Baltimore, MD). The effluent was following chromatography was divided 1:1 between the MS and the olfactometry port. The GC conditions were as follows: 0.5 µL sample was injected via air sandwich technique into the inlet which was held at 250 °C set to split-less mode, helium carrier gas was at a constant pressure of 26 p.s.i. or 180 kpa and the GC oven temperature program was as follows: initial conditions 40 °C held for 2 minutes, followed by a 7 °C/min ramp until 250 °C which was held for 10 minutes. Flavor dilution was carried out as demonstrated in previous studies (136). Each sample was diluted by half-volume in dichloromethane until the dilution had been carried out until a concentration of 128^{th} of the original extraction had been achieved. The largest dilution at which each compound was detected was defined as the flavor dilution (FD) value. Each dilution was analyzed a minimum of three times by two panelists (Both male, both age 25). Compound identification was performed using mass spectral data, odor descriptors and LRI of authentic compound. LRI values were calculated using an n-alkane ladder. Authentic standards were used when available.

Gas Chromatography-Mass Spectrometry Identification and Quantitation

GC-MS analysis was performed using a GC (Agilent 7890, Santa Clara, CA, USA) coupled to a time of flight (TOF) MS (LECO Pegasus 4D, St. Joseph, MI, USA). The isolate was analyzed on two alternate column chemistries namely, DB-5 and DB-Wax. For the DB-5 analysis analogous column and oven conditions were used as reported in "GC-O-MS: AEDA" above. For the DB-Wax (60 m x 0.25 mm i.d. x 0.25 µm film thickness, Agilent Technologies) the GC conditions were as follows: 0.5 µL was injected

into an inlet heated to 250 °C and the GC oven temperature program was as follows: initial conditions 40 °C followed by a 5 °C/minute ramp to 250 °C and then held for 10 minutes.

Quantitation was carried out using a five-point calibration curves for each of the 18 compounds in the following concentration ranges (µg/kg) listed, hexanal (50-800), 2-methyl-pyrazine (55-880), 2,3-dimethylpyrazine (51-815), 2,5-dimethylpyrazine (54-860), methylthaizoline (61-975), 2-pentylfuran (43.5-775), 2-ethyl-3,5-dimethylpyrazine (52.5-840), 3-hydroxy-2-methyl-4H-pyran-4-one (44-700), 2-methylphenol (60-965), 2-acetyl-2-thiazoline (61-975), 2,4-decadienal (92.5-1480), 2-methoxy-4-vinylphenol (438-7000), 4-hydroxy-3-methoxybenzaldehyde (612-10000), all curves were had high linearity ($r^2 > 0.98$ for all compounds). The quantitation was then checked using standard addition against puff samples prepared as previously described with the exception that compound standards were added to the DCM solvent and the peak areas were monitored for change.

Sensory Evaluation

Aroma of the corn puffs was evaluated by a panel of 12 trained panelists (4 male and 8 female ages 22-32) from the University of Minnesota Department of Food Science and Nutrition (St. Paul, MN). Sensory experiments were carried out in a climate controlled room. A descriptive analysis (DA) approach was taken where descriptors were compared to corn puff samples and recombination samples. Descriptions standards and the samples were presented to the panelists in amber glass containers. The recombination samples were prepared using corn starch with added concentrations of the compounds

identified as causing differences in the refined and whole grain corn puff samples seen in Table 1. Panelists were asked to ortho-nasally asses the intensity of six aroma descriptors on a 0 - 15 pt. scale with 0 being not noticeable and 15 being intensely detectable. The descriptors were compared with representative food samples where, dry uncooked Bob's Red Mill Steel Cut Oats represented the oxidized aroma, Bergen Unsalted Lightly Roasted Almonds represented the roasted aroma, Organic Valley Whole UHT Milk represented the cooked aroma, Toasted Wonder Bread™ represented the toasted aroma, Nilla® Wafers represented the vanilla aroma and Old Dutch Resturante® Style Yellow Corn Tortillas Chip represented the corn chip aroma. Prior to the descriptive analysis sessions, the panelists were trained in recognition of the description as well as using the intensity scale. Lastly, the data were analyzed using analysis of variance and Tukey's test with a probability of $p \leq 0.05$. All samples and recombination samples were evaluated in duplicate session.

RESULTS AND DISCUSSION

To investigate the aroma profiles of food products and obtain useful information that allows for meaningful comparison, a robust and comprehensive volatile extraction protocol is needed. Historically non-polar solvents such as diethyl ether and dichloromethane (DCM) have been used for extraction of volatile aroma compounds (83). These solvents are thought to have generally good affinity for odorants and due to their low boiling points, they can be concentrated via distillation for GC analysis. Although these solvents have been proved effective for volatile compound fingerprinting (101,106), they are not without limitations. One example of a limitation is non-polar

solvents will extract non-flavor non-polar food constituents such as fat that can complicate analysis. The removal of unwanted constituents requires rigorous and costly clean-up steps and might result in loss of important analytes. Non-polar solvents can also suffer from reduced extraction efficiency for certain compounds thus can hinder the isolation of a comprehensive aroma profile. Further exploration of suitable and comprehensive extraction protocols is warranted.

For this work a multi-solvent extraction protocol was compared to a traditional solvent extraction. The multi-solvent system protocol incorporated the use of a more polar solvent, methanol (MeOH), as a primary extraction solvent followed by a DCM solvent inversion, this method was referred to as MeOH-DCM. The traditional solvent protocol utilized DCM for the extraction and was followed by clean up steps to remove fat and other non-volatile components. The goal of the comparison was to examine the extraction efficiency of each protocol. The extraction protocols were carried out in triplicate. The solvents were spiked with internal standards to monitor extraction reproducibility. The resulting extracts were compared using the GC-O to examine the number of aroma-active regions detected at an equivalent fold sample concentration. The MeOH-DCM derived extract contained 111 aroma active regions compared to 86 aroma active regions in the traditional solvent extract. Hence the MeOH-DCM protocol was chosen for use of the subsequent GC-O-MS analysis and quantitation. The increase in extraction efficiency was attributed to the chemical and physical properties of methanol. The small molecular diameter of methanol as well as its polar nature would be expected to have more interactions with the hydroxyl groups of the starch matrix. These

interactions can potentially increase solvent penetration and allow for more efficient interaction with aroma active compounds.

Following MeOH-DCM extraction of WGP and RFP samples, the aroma differences were characterized through comparative GC-O-MS AEDA analysis. AEDA allows for the determination of odor active regions across the food extract after serial dilutions. The serial dilutions produce what are referred to as 'Flavor Dilutions' (FD). FDs give insight into differences as a compound might be present in later flavor (more dilute) dilutions in one sample than another. The presence in a later dilution indicates a higher concentration and potential differences in impact on aroma (136). The technique can be applied to multiple samples as used as a screening process that reveals unique compounds or compound concentrations in the form of FDs between the two samples. In this case the two samples were RFP and WGP.

To characterize the main differences in the aroma profiles of extruded puffs made from whole grain versus refined corn flour, upon completion of AEDA, key odorants were selected if the odorants had an initial FD values ≥ 16 and differed by an FD value ≥ 2. Based on these criteria, 13 key odor compounds were identified hexanal, 2-methyl-pyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, methylthiazoline, 2-pentylfuran, 2-ethyl-3,5-dimethylpyrazine, 3-hydroxy-2-methyl-4H-pyran-4-one, 2-methoxyphenol, 2-acetyl-2-thiazoline, 2,4-decadienal, 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde (shown in Table 2.1). Interestingly, all compounds except for 2,5-dimethylpyrazine had a higher FD value in WGP extracts. All odorants observed have been previously identified in studies on processing impacts of aroma in extruded corn

products (102, 79). The influence of flour type on their generation and their individual impact on the aroma profile of WGP and RGP however was not previously known. Further quantitative analysis of the 13 compounds was conducted and the results shown in Table 2.2. In agreement with the FD values reported in Table 2.1, of the 13 compounds; 5 Maillard compounds, 2-methyl-pyrazine, 2,3-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-hydroxy-2-methyl-4H-pyran-4-one, 2-acetyl-2-thiazoline were found to be statistically higher in concentration in the WGP samples while 2,5-dimethylpyrazine was found to be statistically higher in the RFP, one Maillard compound, methylthiazoline was not found to be statistically different between the two puffs. Two phenolic compounds; 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde were also found to be statistically higher in the WGP while 2-methoxyphenol was not found to be significantly different. Three Lipid oxidation compounds; hexanal, 2-pentylfuran, 2,4-decadienal were not found to be statistically different.

The identified compounds are known products of three reaction pathways; lipid oxidation, the Maillard reaction, and phenolic degradation. These three reaction pathways are also known to have interplay thus, each pathway can affect product generation of the others (72, 92, 93). The formation of the identified aroma compounds was greater for all three formation pathways in the puff with whole grain flour. These results can be explained by compositional differences between whole and refined grains. In the case of the refined corn flour the grain is milled the bran/gram is removed and leaving the starchy endosperm, while the whole grain all components of the kernel remain. Because

of milling removing bran/gram, refined corn flour has a reduced concentration of lipids and proteins (91) compared to whole grain. Lipids and proteins are both known precursors of the reaction pathways responsible for the formation of the identified aroma compounds. Akin to Le Chatelier's principle, changes in the concentration of these initial reaction precursors can have an impact on the products of reactions.

Amino acids are precursors that have been shown to have impacts on reaction pathways. For instance, an increased ratio of amino acids to sugars has also been shown to increases rates of Maillard reaction (107). Protein and amino content has been reported to be higher in whole grain corn flour compared to refined corn flour (76, 91). It is feasible that an interaction occurs in corn during extrusion processing results in a higher rate of formation and thus concentration of Maillard compounds as there are more reactants. Amino acids are very influential for the progression of Maillard reaction pathways[DP2] (114), through the interaction with reducing sugars resulting in compounds like 2-acetyl-2-thiazoline and 2-ethyl-3,5-dimethylpyrazine which showed concentrations 2.2 and 2.1-fold higher in WGP when compared to RFP. Additionally, the lower initial concentrations of amino acids in refined flour might influence the interactions with sugars in the RFP and could potentially favor specific reaction products in the Maillard pathway that favor the formation of 2,5-dimethylpyrazine.

A higher concentration of phenolic acids (bran material) in the whole grain flour (107). For instance, increased ferulic acid in whole grain flours can also result in key differences in the odorant profiles between refined products and ones made with whole grains (92). In whole grain corn flour, has approximately 26,000 µg/g of ferulic acid

while the endosperm contains approximately 170 µg/g (137). Due to the increased amount of ferulic acid, as expected, two ferulic acid degradation products 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde (94), which respectively have clove and vanilla aroma descriptors were higher in concentration in the WGP and were suggested to contribute to the aroma differences when compared to RFP. Thus, the impact of corn flour composition on flavor formation was evident since as elevated amounts of the ferulic acid are present in the whole grain flour; which is remove from refined flour.

To draw further insight regarding the impact of quantitative differences of observed odorants of the extruded puff (Table 2.2) on the flavor profile, sensory descriptive analysis was conducted on both the WGP and RFP samples (shown in Figure 2.2) as well as aroma recombination models (shown in Figure 2.3). This was an important step in evaluating if the quantitative findings equated to perceptible sensory changes. Descriptive analysis (DA) was employed to identify the main sensory attributes of the products, develop a lexicon, for a direct sample comparison.

In general, the sensory results agreed with AEDA and quantitative data. The perceived intensities of the aroma descriptors were generally higher in WGP versus RFP. For the WGP sample, the higher reported mean intensity of cooked, corn chip, roasted and toasted notes (Figure 2.2) corresponded to the increased concentration of the Maillard derived compounds (Table 2.2). Furthermore, lipid oxidation notes were not shown to be statistically different in the DA data (Figure 2.2). This finding which corresponds to the lipid oxidation products, hexanal, 2-pentylfuran and 2,4-decadienal

which were not found to be statistically different between WGP and RFP during quantitation (Table 2.2).

In addition to the direct comparison of corn puffs, recombination samples containing compounds added to corn starch at concentrations reflecting the quantitative results (Table 2.2) were also examined by the DA panel (see Figure 2.3). Overall, results of the recombination DA agreed with DA analysis of corn puffs which found toasted, roasted, corn chip and cooked attributes to have higher mean intensities in whole grain recombination samples (Figure 2.3). While like the DA of puffs the lipid oxidation attributes did not reflect a significant difference in mean intensity between the whole grain and refined recombination samples. This further validated the importance of the identified compounds and quantified compounds (Table 2.2) and demonstrated their impact on the differences in the aroma profiles of the extruded RFP and WGP (Figure 2.2). Interestingly phenolic compounds; 2-methoxy-4-vinylphenol and 4-hydroxy-3-methoxybenzaldehyde that have descriptors like clove and vanilla descriptors did not have sensory significance. This is of note as the compound had concentration increases of 4.3 and 2.9 in WGP compared to RFP which would lead one to assume sensory importance. This finding illustrates the importance of sensory validation of aroma studies to define whether the concentration differences equate to aroma perception differences.

This study found that Maillard reaction products were higher in concentration and important drivers of the aroma perception of differences between WGP and RFP. While, lipid oxidation and phenolic degradation reaction products were not found to be influential in aroma perception. Historically the aroma attributes that Maillard

compounds are associated with; roasted, toasted, corn chip and cooked have been shown to be viewed as positive (101). Given the higher concentrations and higher intensity of aroma attributes associated with Maillard compounds it is difficult to determine from this study what this increase means for consumer preference. Another possibility is that other flavor inputs such as taste compounds might be the more important in influencing consumer preferences for refined grain products and avoidance of whole grains products (71). Due to this the following chapter of this dissertation will examine factors that influence key bitter compound in aroma corn puffs.

In summary, Maillard browning was shown to be the primary driver of aroma differences between the refined and whole grain puffs flour formulation. These finding show how flavor can be altered by the composition of flour that is used for extrusion processing. The work also provides insight into the flavor formation pathways to a more desirable product. Further elucidating the aroma formation mechanistic pathways can facilitate flavor optimization.

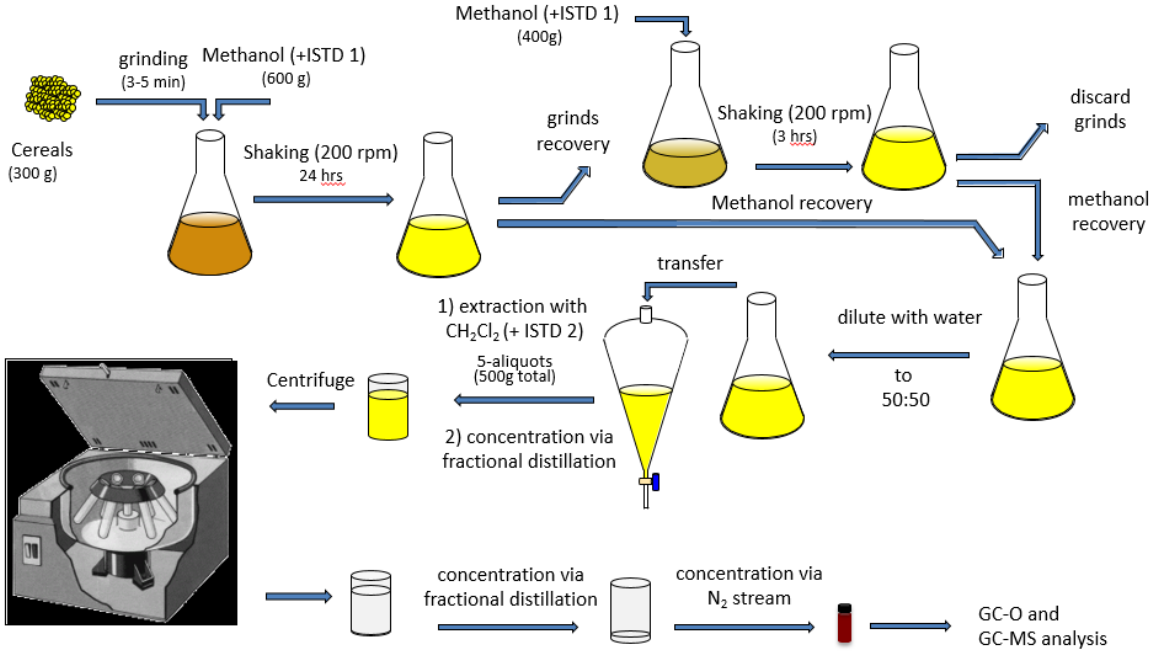


Figure 2.1: Volatile Extraction Protocol

Table 2.1: Odorants with a FD ≥ 16 and a difference ≥ 2 between the refined corn puffs (RFP) and whole grain corn puffs (WGP) (136)

Compound[b]	Descriptor[c]	LRI		FD Factor[a]		FD Ratio (WGP/RFP)
		Wax	DB-5	RFP	WGP	
hexanal	Green	1084	801	32	128	4
2-methyl-pyrazine	Roasted	1176	827	32	128	4
2,3-dimethylpyrazine	Roasted	1240	911	32	128	4
2,5-dimethylpyrazine	Roasted	1253	912	32	16	0.5
methylthiazoline	Roasted/Toasted	1436	933	64	128	2
2-pentylfuran	Bean	1240	993	32	128	4
2-ethyl-3,5-dimethylpyrazine	Roasted	1457	1081	32	32	2
3-hydroxy-2-methyl-4H-pyran-4-one	Caramel/Toasted	1955	1087	64	128	2
2-methoxyphenol	Smokey	1872	1088	64	128	2
2-acetyl-2-thiazoline	Popcorn/Corn Chip	1772	1103	32	64	2
2,4-decadienal	Oxidized	1815	1312	32	64	2
2-methoxy-4-vinylphenol	Clove	2189	1322	64	128	2
4-hydroxy-3-methoxybenzaldehyde	Vanilla	2589	1410	16	64	4

[a]Flavor dilution based on the average of two panelists, [b]compound positively identified (LRI, MS and authentic compound), [c]Odor described at sniffing port during GC-O

Table 2.2: Quantification of Select Aroma Compounds in Refined (RFP), Whole grain (WGP) extruded corn puffs. Different letters (a, b) indicate a statistically significant difference between samples (n = 3 and α = 0.05), *aroma threshold concentration (µg/kg) in water (137)

Compound	Concentration (µg/kg)		Concentration Ratio (WGP/RFP)	Aroma Thresholds in Water*
	RFP	WGP		
hexanal	436[a]	470[a]	1.1	5
2-methyl-pyrazine	292[a]	363[b]	1.2	60000
2,3-dimethylpyrazine	280[a]	653[b]	2.3	2500
2,5-dimethylpyrazine	141[a]	100[b]	0.7	200
methylthiazoline	137[a]	147[a]	1.1	10
2-pentylfuran	153[a]	183[a]	1.2	6
2-ethyl-3,5-dimethylpyrazine	124[a]	260[b]	2.1	1
3-hydroxy-2-methyl-4H-pyran-4-one	321[a]	370[b]	1.2	3500
2-methoxyphenol	297[a]	317[a]	1.1	50
2-acetyl-2-thiazoline	168[a]	377[b]	2.2	3
2,4-decadienal	243[a]	293[a]	1.2	0.7
2-methoxy-4-vinylphenol	843[a]	3600[b]	4.3	10
4-hydroxy-3-methoxybenzaldehyde	1218[a]	3517[b]	2.9	200

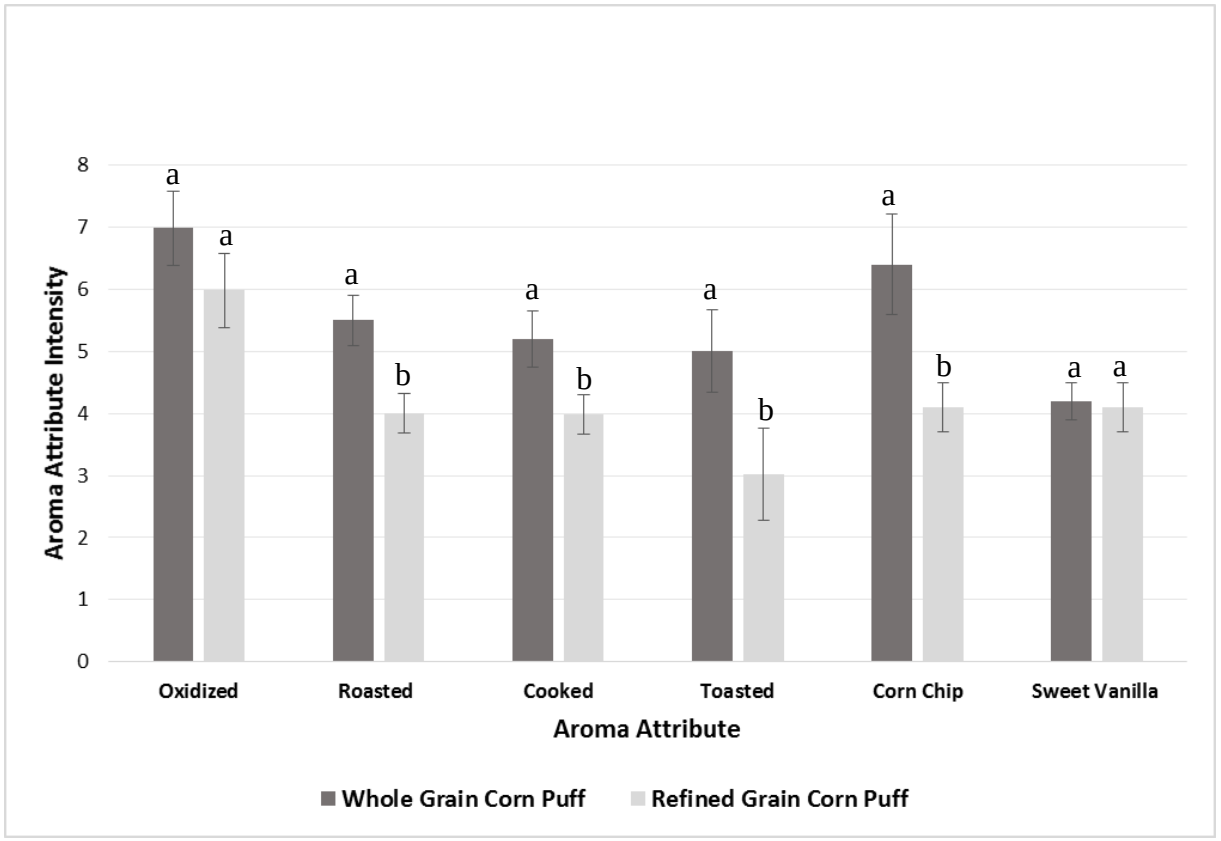


Figure 2.2: Comparison of whole grain puff (■) and refined grain puff (□) aroma attribute mean intensity scores. Attribute that have the same letter code were not significantly different between whole grain and refined puffs. Panels consisted of 12 panelists and the sensory testing was carried out in duplicate. ($p < 0.05$)

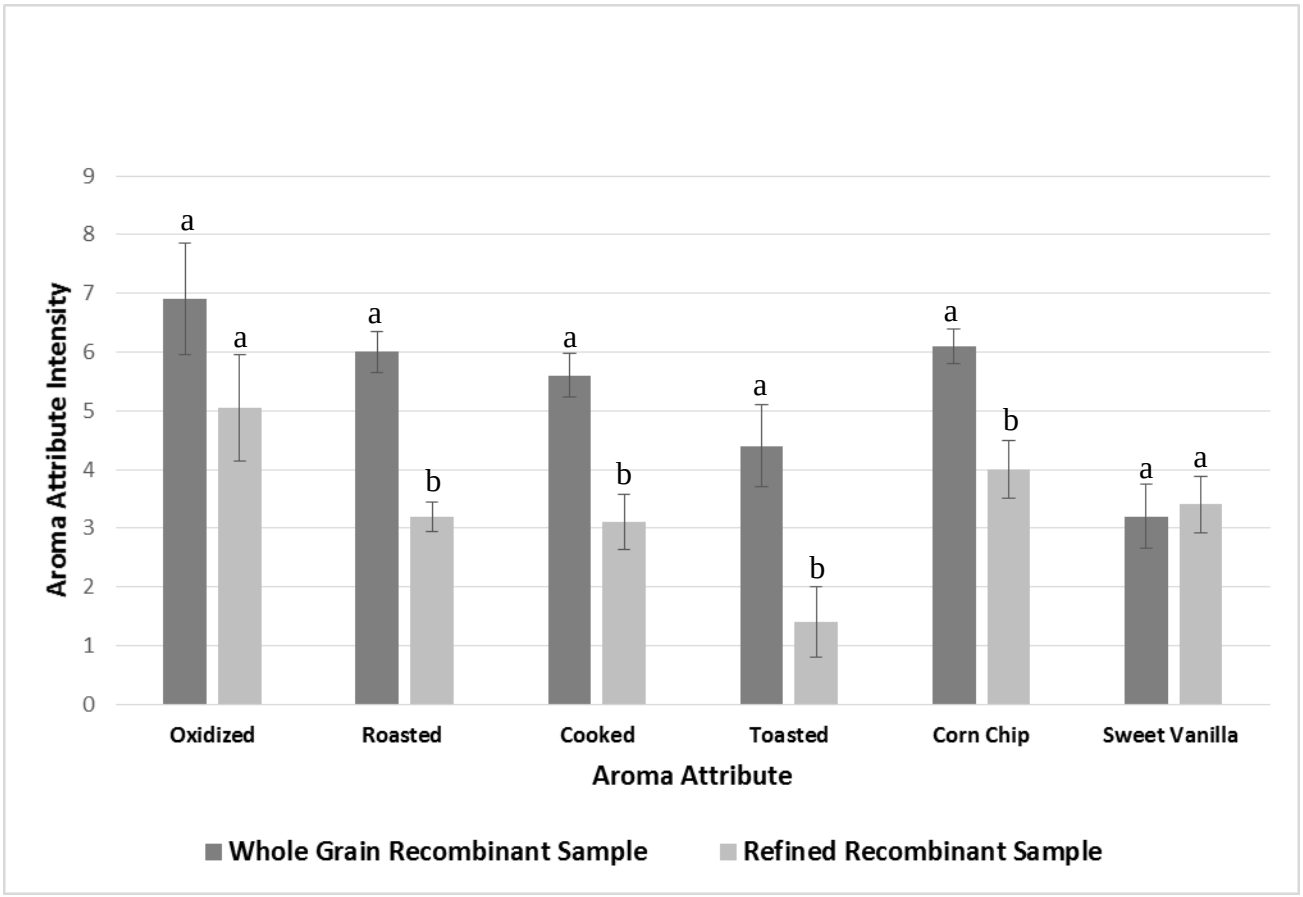


Figure 2.3: Comparison of whole grain difference recombinant (▓) and refined grain difference recombinant ( ) aroma attribute mean intensity scores. Attributes that have the same letter code were not significantly different between whole grain and refined puffs. Panels consisted of 12 panelists and the sensory testing was carried out in duplicate. ($p < 0.05$)

Chapter 3: Investigation of the Liberation of Chaenorpine in Extruded Corn Puffs

SYNOPSIS

The influence of pre-heat flour treatment, storage of whole grain flour, and product formulation on the concentration of the bitter compound, chaenorpine in an extruded whole grain corn puff were investigated. Pre-thermal treatments (60, 80 and 100 °C for 2, 5 and 10 min.) to the flour or storage of the flour (25-35 °C for 3 months) prior to extrusion reported no major changes in the concentration bitter compound in the extruded puffed samples. However, product formulation with higher pH was found to significantly reduce the chaenorpine concentration in the extruded product. The reducing effect was hypothesized to be the result of the pH increase exerted by TSP. Thus, a pH modification study was employed to determine if chaenorpine levels were reduced via a pH induced mechanism. Samples with pH of 5, 6, 7, and 8 were processed via simulated extrusion thermal profile (SETP) as well as pilot scale extrusion. The study revealed that higher pH (7 and 8) processing conditions resulted in significantly lower levels of chaenorpine in the product samples. In addition, sensory descriptive analysis supported the analytical data, indicating a positive correlation between chaenorpine concentration and perceived bitterness intensity.

INTRODUCTION

The consumption of whole grains due to the high content of fiber and vitamins, has been linked to lower rates of chronic conditions such as coronary heart disease, obesity and a decreased risk of colo-rectal cancers (136, 119). Despite increasing consumer awareness regarding the health benefits associated with whole grains, consumption remains low. Most Americans do not meet the USDA recommended 48 g/day (139). Rates have historically been low, based on the USDA recommendations published in 2015 only 20% of the population was estimated to be consuming the recommended amount of whole grains. Despite short comings in consumption there is interest in whole grains, per the Whole Grains Council survey data from 2015 indicating that 62% of respondents self-reported choosing whole grain at least half of the time and of that 62% approximately half or 31% self-reported choosing whole grains "nearly always". Despite this there is still considerable room for growth in consumption.

Although whole grain options are available in the marketplace, consumption has been limited by challenges with negatively impacts including the convenience, cost, and sensory characteristics (135). Many factors account for the observed gap between recommended and actual dietary intake of whole grains including flavor attributes (118) such as excessive bitterness and oxidative aromas which become major limiting factors especially among children (137). A food with whole grain options and a wide acceptance and a large market share, per the Mintel report (129), is breakfast cereal. Mintel consumer reports showed that families with children consume more breakfast cereal and it is estimated that 96% of children consume ready to eat extruded breakfast cereals (129).

Historically the methods that have been used breakfast cereal to overcome negative flavor attributes have been to use additional sugar or salt to mask off-flavors. These methods go against the current trends of low-sodium and low-sugar formulations. In order to overcome the flavor challenges that whole grain products present, while adhering to current market trends a comprehensive understanding of flavor development in whole grain foods is necessary.

Previous work focused on flavor development in extruded products has largely centered on surveying the aroma compounds produced by altering processing parameters like heat, moisture, and feed rate (122,124,131,133). This work has provided information regarding aroma development and the impact of processing on the aroma of extruded puffs. However, there has been very limited work performed on the factors that influence the taste attributes such as bitterness in extruded corn products.

Recent work examining bitter compounds in extruded corn puffs was carried out by Zhang and Peterson (141). The study aimed to identify the bitter markers responsible for the differences in bitterness between refined and whole grain corn puffs. The study identified L-tryptophan, chaenorpine, N^5,N^{10}-Di-[E]-p-coumaroyl-spermidine, N^1,N^{10}-Di-[E]-p-coumaroyl-spermidine and terrestribisamide as the main compounds contributing to bitterness of corn puffs, with their levels in whole grain corn puffs being respectively; 2.5-, 3-, 2.5- and 2.4-fold higher when compared to refined corn puffs. Following the identification of the bitter compounds a sensory study was carried out to establish the impact of each compound on bitterness perception. The sensory study found Chaenorpine to be the primary contributor to bitterness in whole grain corn puffs.

Given the general negative impact of bitterness on food flavor and consumption[DP3], this study aims to build on prior work (141) and investigate the impact of flour pre-processing conditions and flour age, as well as product formation on the concentration of chaenorpine in extruded corn puffs. The overall goal of this study is to provide insight and facilitate the development of processing and ingredient guidelines for the creation of high flavor quality whole grain products.

METHODS

Bench Scale Simulated Extrusion Temperature Profile (SETP) Processing

A simulated benchtop processing method was developed based on previous work reported by Kokkinidou and Peterson (128) to simulate high temperature processing with gram sample quantities. In brevity, a CTC Analytics PAL Heated Agitator (CTC Analytics AG, Zwingen, Switzerland) was used for the simulated thermal processing treatment. Briefly, 1 g of whole grain flour (Bunge, St. Louis, MO, USA) and 0.14 g water was placed in 20 mL headspace vials (Restek, Bellefonte, PA, USA). The vials were placed in a chilled vial holder and held there until they reached an internal temperature of 12 °C. Vials were transferred to an agitator (200 °C) and held for 95s to allow the sample to reach a final temperature of 150−151 °C (simulated extrusion temperature profile – SETP) and was subsequently returned to the cooler. The time-temperature profile of the simulated bench scale processing was designed to mimic the commercial heating profile typical for corn product extrusion. To verify that the process

was matched the targeted heating profile, temperature was monitored by a thermocouple probe placed in the corn flour sample throughout the SETP process.

Pre-Heat Flour Treatment. Corn flour (1 g) and 0.14 g water was placed in to a 20mL headspace vial and heated to 60, 80 or 100 °C for 2, 5, and 10 minutes via the bench scale heat treatment setup. Subsequently vials were transferred to a chilled vial holder set to 12 °C. Samples were then subjected to SETP treatment or removed for storage at -80 °C prior to further analysis.

Ingredient Formulation Analysis: Ingredient interactions and their impact on the levels of Chaenorpine in the final product were explored using the SETP

protocol. Trisodium phosphate (TSP) (Sigma Aldrich, Milwaukee, WI, USA), NaCl or salt (Sigma-Aldrich), calcium carbonate ($CaCO_3$) (Fisher Scientific, Fairlawn, NJ), sucrose (Sigma-Aldrich), glyoxal (Sigma-Aldrich) were added to 1g of flour in the 20mL headspace vials at 1% (w/w) with the exception of glyoxal which was added at 1 and 5% (w/w) to the flour. The samples were then subject to pre-heat treatment, SETP or a combination of both as described above.

Analysis of Flour with Modified pH. A pH modification study was carried out in order to further understand the impact of pH on the concentration of chaenorpine. Whole grain corn flour (1g, Bunge) was placed in a 20mL headspace vial (Restek Corporation, Bellefonte, PA) and was subjected to a 2 minutes pre-processing treatments at 60, 80 and 100 °C followed by the SETP treatment. Prior to the pre-processing treatments and SETP treatment the pH of the corn flour was modified to pH 5, 6, 7, and 8 using solutions of

phosphoric acid and sodium hydroxide (Sigma Aldrich). All samples were produced and analyzed in triplicate.

Analysis of Aged Flour. Previous work by Shoba, (134) has suggested a significant pH change during storage of flour thus, a shelf-life study was performed to monitor the effect of aging on pH and the content of chaenorpine. Flour was stored at 25 °C and 35 °C for 3 months in sealed HDPE bags and each bag contained 50 g. At 1, 2 and 3 months, two bags of flour were removed flour was removed from one bag and the pH was taken the other was left sealed. Following obtaining the pH both bags were stored at -80 °C until the end of the storage period. At the end of the storage period pH, Chaenorpine content were measured. Subsequently, flours were subjected to SETP (method outlined above) and pH and Chaenorpine content were additionally measured in the SETP product.

Determination of product pH

The pH of all samples was measured according to the AACC international 02-52.01 hydrogen ion activity (pH)-electrometric method using a VWR SympHony pH meter (VWR, Radnor, PA, USA). The method was adapted from (123) where 1.5 g of samples were ground, weighed, and agitated with 10 g of NanoPure™ (ThermoFscher Scientific, Waltham, MA) water until a suspension was formed. The suspension was allowed to rest for 30 minutes and the supernatant was then collected and the pH was immediately determined using an electrode that had been calibrated against known standard solutions. The pH measurements were performed in triplicate per treatment.

Pilot Plant Extrusion Processing

Extrusion conditions were designed to yield uniform air cell structure throughout each puff (122, 126, 129) Briefly, extrusion processing was carried out using a Buhler DNDL-44 twin screw extruder (Buhler Inc., Plymouth, MN, USA). Two formulations were designed: the refined corn flour formulation and the whole grain corn flour formulation.

The refined corn flour formulation consisted of 50 kg (100%) Bunge whole grain corn flour with, 14% added water, 1% trisodium phosphate, 1% calcium carbonate, and 1% sodium chloride. A low work screw was optimized the hydration condition with the primary work load carried out by the final elements.

The mixed flour was added to the extruder and processed with the following extrusion parameters: computer controlled shaft speed of 350 rpm, a measured die pressure of 10.1 ± 0.5 bar, die temperature of 150 ± 1 ˚C, material throughput of 50.8 ± 0.1 kg/hr with 7 kg/hr water and a cutter speed of 1200 rpm resulting in 1/4 inch puffs. Due to differences in the physical and chemical characteristics of the refined and the whole grain flour mixes, the refined corn flour formulation showed an increased shaft torque of 224 NM versus the whole grain corn flour formulation of which had a shaft torque of 215 NM; the specific mechanical energy for refined corn flour formulation was 164 kw/h while the whole grain corn flour was 159 kw/h.

Other parameters were constant across both formulations. The puffed product was collected and dried on a liquid air bed and stored in high density polyethylene bags at -40 ℃ for later analysis.

Extrusion Ingredient and pH Modification. In addition to the initial extrusion study a series of modified formulations were produced using the extrusion conditions listed

above. The modified formulations used 50 kg whole grain corn flour (Bunge), 14% (w/w) water, 1% (w/w) Tri-sodium phosphate (TSP), 1% (w/w) calcium carbonate and 1% (w/w) sodium chloride (NaCl) as the base formulation. Calcium carbonate and TSP are both buffering salts and alter pH.

Each ingredient was removed or run as the only ingredient in addition to water and whole grain corn flour. The experiment was designed using full factorial design to explore the impact of each ingredient and the resulting sample formulations are shown in Table 1. In addition to the ingredient modified products a series of products were prepared to examine the effects of pH on the final concentration of chaenorpine. Products were prepared using whole grain corn flour (Bunge) and 14% (w/w) moisture and sample formulations were completed by adding on of the following; 0.15% (w/w) by weight citric acid (Sigma-Aldrich), 0.75% (w/w) citric acid and 1% TSP, and 0.5% (w/w) 1M phosphoric acid (Sigma-Aldrich). Extrusion products were stored at -40 ℃ prior to further analysis.

Sample Extraction Protocol of Chaenorpine Analysis

To extract chaenorpine for subsequent analysis a method designed by Zhang and Peterson (141) was modified to meet the lower sample size and higher throughput needs of this study. For corn flour and SETP processing products 1 g of sample was ground in a blender for 2 min, and then extracted twice using 75% ethanol-25% water solution (3 mL) at room temperature for (3 h). The solvent extracts resulted from the two extractions were pooled and subsequently centrifuged using a Beckman-Coulter Avanti J-E (Beckman-

Coulter, Brea, California, USA) at 4000 x g for 10 min. Following centrifugation 4.5 mL aliquots of the solvent were placed into test tubes which were then subject to solvent removal using a centrifugal evaporator GeneVac™ EZ-2 (SP-Industries, Warminster, PA, USA) until dryness. Following evaporation, samples were dissolved in 2 mL of NanoPure™ (ThermoFscher Scientific, Waltham, MA, USA) and then underwent solid phase extraction (SPE) for further clean-up and concentration. SPE was performed using 3M Empore™ C-18 Standard Density SPE cartridges (3M, St. Paul, MN, USA) that were preconditioned with water followed by 100% methanol. The analyte of interest was eluted from the cartridge using a 3 mL 70% methanol solution. Following the SPE step of the extraction the eluent was collected and subjected to a final 0.22µ filtration. Samples were then stored at -20 °C until LC-MS-MS analysis.

Recovery efficiency of chaenorpine with the above was calculated by a standard addition technique, using samples spiked with known amounts of chaenorpine. Food grade purified Chaenorpine was recovered from corn flour (Bunge) using the two-dimensional fractionation method outlined fully by Zhang and Peterson (141). Briefly, the corn puffs were extracted using a 95% ethanol -5% water solution. Next 40 mL aliquots were filtered then injected on to an HPLC and separated first on a preparative RP C-18 column (21.1mm x 250 mm pursuit 5, Varian, USA). The injection was fractionated in 1 min. intervals. The fractions from each injection and separation containing chaenorpine were then pooled, the ethanol was evaporated. The sample was freeze dried. Following the freeze-drying step the sample was reconstituted in NanoPure™ water. A second-dimension fractionation followed using a Zorbax Bonus-RP Column (21.1mm x 100 mm x 5µm) was carried out.

This step is done to achieve purification of chaenorpine Following sample injections, the fractions were collected and pooled. The pooled fractions were then evaporated and freeze dried twice to remove water and solvent. The resulting powder was checked for purity using the LC quantitation method listed below. The purified chaenorpine was then stored at -80 °C in seal amber vials. Total chaenorpine was calculated by using a standard curve and recovery was found to be in the range of 87% ±3%, which indicates that the isolation method had good reproducibility.

Quantification of Chaenorpine LC-tandem mass spectrometry

Quantitative analysis of Chaenorpine was conducted using a Acquity UPLC system connected to a Waters Quattro Premier XE™ Triple Quadrupole mass spectrometer (Waters, Milford, MA, USA). Two microliters (2 µL) of the extract was injected on a UPLC C18 BEH 1.7 µm (2.1 mm x 50 mm) column kept at 30 ºC. Chromatographic separation was performed using water with 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B), the flow rate was set to 0.3 ml/min and the following gradient was employed: solvent B was increased from 5 to 95% from 0 to 6.45 min, 95% Solvent B was decreased beginning at 6.45 to 7.45 min to 5% solvent B, initial conditions were then maintained from 7.45 to 9 min. The Quattro Premier XE™ MS was equipped with an electrospray probe for ionization of the eluents. The system was set to and positive ion mode with a source temperature 110 °C; a desolvation temperature of 350 °C; and a capillary voltage 30 v. Data were collected/obtained in multiple-reaction-monitoring (MRM) mode. The ion transitions, cone voltages (CV) and collision energies (CE) for

chaenorpine and the internal standard (methylparabens) chaenorpine were m/z 493 to 265, CV: 30v, CE: 25v. Methylparaben (1 M) was used as internal standard: m/z 153 to 121. All samples were injected and analyzed in triplicate. Quantitation was performed using a five-point standard curve of chaenorpine and results were evaluated by a pairwise comparison statistical analysis with level of significance was set at 0.05.

Sensory analysis

In order to determine the if the changes to the concentration of chaenorpine resulted in changes to bitterness perception a descriptive sensory panel was used to rate the bitterness intensity of the samples. The panelists tasted corn puffs that were produced at the University of Minnesota in the Joseph J. Wartheson Pilot Plant. The panel consisted of twelve experience panelists (4 male and 8 female, ranging from 21 to 45 years old) were recruited from The Ohio State University. Panelists participated in three 1hr training sessions to familiarize themselves with the product and to define the tasting protocol. Each sample cup had a randomized 3-digit code and contained 10 corn puffs (approximately 2 g). Panelists were instructed to place the entire sample in their mouth, masticated until sample was completely hydrated and rate the perceived bitterness. Panelists then rated the bitterness intensity on a 10cm line scale anchored with three intensity references (1 = 0.025%, 2 = 0.05%, and 5 = 0.15% caffeine w/v). Samples were evaluated in duplicate, panelists participated in 2 sessions occurring over the period of 1 day. Water and unsalted crackers were used as palate cleaners and panelists were

instructed to wait at least 1 minute in between samples. All data was collected using Compusense Cloud Software (Compusense Inc., Guelph, Ontario, Canada).

RESULTS AND DISCUSSION

Understanding how bitterness is impacted by processing and formulation parameters provides a basis to improve palatability of whole grain products. Ultimately the understanding will help deliver foods that promote and support public health. To better understand bitterness development in extruded whole grain corn systems, factors influencing chaenorpine concentration, the primary compound contributing to bitter perception in extruded corn puffs (140), were examined. Chaenorpine was recently identified and quantified in extruded corn puffs, it was found to be three times higher in concentration in whole grain corn puffs (0.3 mg/g) when compared to refined corn puffs (0.1 mg/g). Additionally, chaenorpine was shown to be degraded during extrusion processing of ready-to-eat cereal, suggesting that optimization of extrusion or formulation could be utilized to mitigate bitterness. The result of the mitigation would improve the flavor quality of whole grain extruded products.

The investigation aimed to understand how processing and commonly used GRAS processing aids or ingredients influence chaenorpine content in the extruded product. The impact of low heat treatments prior to simulated extrusion temperature profile (SETP), flour storage (3-month shelf life study), ingredient knockout extrusion pilot production, as well as pH modification in SETP and pilot scale extrusion were investigated. Rational for each area of study is discussed below.

The initial bench scale investigation examined the influence of a pre-processing heat treatments of the flour on the resultant chaenorpine content. Pre-processing heat was explored due to the previously reported reducing effect of extrusion processing on the amount of chaenorpine present in WGP when compared to raw flour (140). It was hypothesized that pre-processing thermal treatment may induce thermal degradation pathways and result in further reduction of chaenorpine in the extruded product. For example, the generation of sugar fragments, during the Maillard reaction such as methyl glyoxal, might react with chaenorpine (degrading it) resulting in a lower the concentration. Three temperatures, 60 °C, 80 °C, and 100 °C and three treatment times, 2, 5, and 10 minutes were selected as investigation points. At these temperatures and moisture content, starch would not be expected to undergo gelatinization (127).

The samples that underwent pre-processing heat treatments as well as pre-processing heat treatment and SEPT are shown in Figure 3.1 and 3.2, respectively. Pre-processing treatments resulted in an increase in the concentration of chaenorpine for some of the treatments. More specifically, pre-processing treatments at 60 °C resulted in 30%, 23%, and 23% increase in chaenorpine, for the two-, five-, and ten-minute treatments respectively, compared to raw flour. A potential explanation for the increase might be liberation of chaenorpine from the flour matrix changes induced by low temperature thermal processing (125). For example, chaenorpine might be bound by ester linkages that are common in corn (140) and liberated by heat and under acid conditions[DP4].

However, at higher temperatures of pre-processing treatments, the effect on the concentration of chaenorpine was not as well-defined. As the time of the pre-processing treatment increased the increase in concentration of available chaenorpine was diminished to the point where it was the same as the flour. Additionally, the 5 and 10 minute 80 °C pre-processing treatment, were not found to be significantly different from raw flour. The 2 minute 80 °C treatment showed a statistically significant change with chaenorpine increasing by 17.5% when compared to raw flour. The lower chaenorpine liberation of high temperatures when compared to the 60 °C pre-processing treatment may be induced by a heat induced mechanism resulting in consumption and/or degradation of liberated chaenorpine. At the higher heat treatment conditions (100 °C) the potential consumption pathway rate was occurring as fast as the liberation over processing time, which could be a potential explanation for the minimal increase observed in the longer five- and ten-minute 80 °C treatments and all three of the 100 °C pre-processing treatments. This effect could also be playing a role in the 60 °C samples as there the two minute samples showed a 30% increase while the 5 and 10-minute processing times resulted in a lower 23% increase of chaenorpine.

To further examine how the changes caused by the pre-processing treatment might impact the final product, samples underwent both pre-processing heat treatment prior to SETP, and the levels of chaenorpine were determined (Fig 3.2). The results suggested that implementation of a pre-processing treatment had for the most part no significant impact on the level of chaenorpine with few exceptions that revealed an increasing concentration trend for, 60 °C for 2 and 10 minutes and 100 °C for 10 minutes.

Thus, the implementation of a pre-processing step did not induce degradation of chaenorpine and would not provide an effective mitigation strategy for the reduction of bitterness. Thus, alternative approaches were explored for their potential to alter chaenorpine content.

Based on the observed liberation of chaenorpine with the pre-heat treatment, this suggested chaenorpine was bound to the flour matrix and liberated by hydrolysis. Hydrolysis reactions would also be expected to be influenced by the product pH. The pH of the product would be affected by typical processing aid ingredients used for extruded cereal products, such as $CaCO_3$, and trisodium phosphate (TSP), thus they were selected for further analysis. Additionally other common ingredients such as sugar and NaCl were investigated. Sugar would provide an accessible carbonyl source to develop reactive Maillard intermediates, like sugar fragments which may degrade chaenorpine. To more directly test the influence of sugar fragments as a mechanism of degradation, the influence of the sugar fragment glyoxal was also studied.

The effect of each ingredient on the final concentration of chaenorpine after SETP treatment is shown in Figure 3.3. No significant differences were observed for the $CaCO_3$, NaCl, sugar and glyoxal treatments, however the addition of TSP or TSP with sugar resulted in a reduced concentration of chaenorpine by 15% in comparison to the flour (control) sample. The ingredients and processing aids examined contribute different qualities to the finished products as well as altering the system during processing. Salt and sugar can alter water absorptivity by the starch gelatinizing polymers due to competition for available water or through other means. For example, salt contributes

ions that can change polymer cross linking and alter the solubility of amino acids and proteins. These changes could potentially impact flavor development by binding or releasing compounds in addition to changing the availability of water, which can impact chemical reactions such as the Maillard reaction. Unlike $CaCO_3$ which favors neutral pH and NaCl which has little direct impact on pH, TSP has the ability to increase the pH of a system. Thus, as a next step, the effect of pH on the levels of chaenorpine was explored. TSP is the conjugate base of phosphoric acid and has a pK_b of 2.23. This characteristic of the compound enables it to be used to raise the pH of a particular system. The pH of the system was selected for further examination. In order to further examine the pH impact on the final concentration of chaenorpine a series of bench scale samples were designed with altered pH.

The pH of samples was adjusted from 6.6 to 5, 6, 7 and 8 via addition of NaOH and phosphoric acid to flour to achieve basic and acidic conditions respectively. These levels were selected as they cover a broad range of pH values that can give insight into basic and acidic pH range and observed ranges in commercially available corn flour (134). Additional processing aids and ingredients were not included in the formulation in order to independently study pH.

The samples with a basic pH showed a significantly lower concentration of chaenorpine when compared to the samples with acidic pH (data shown in Figure 3.4). To provide context to this change in the concentration of chaenorpine, samples that were modified to pH 6 had a concentration of 0.57 mg/g while samples that underwent the same SETP at pH 7 had a chaenorpine concentration of 0.28 mg/g. The difference in

concentration equates to a nearly two-fold increase in chaenorpine at an acidic pH. However, the change from acidic to basic pH region seems to be the major driver in altered chaenorpine concentration as the change from pH 5 to 6 or 7 to 8 was not as pronounced.

Given that the SETP benchtop system did not fully mimic all mechanical factors of extrusion processing such as shear and pressure, a pilot scale extrusion study was subsequently conducted to examine the effect of pH on the levels of chaenorpine in extruded corn puff products as influenced by ingredient formation, which also impacted the product pH.

The different sample formulations analyzed are shown in Figure 3.5. The pH of the samples was not adjusted in these set of experiments but samples with different pH values resulted due to formulation differences. The samples were formulated to systematically include and exclude ingredients in order to gain further insight regarding their impact on the final concentration of chaenorpine in extruded corn puffs. As seen in Figure 3.5 the sample 'Flour, TSP' sample had the lowest concentration of chaenorpine followed by 'Flour, TSP, $CaCO_3$' sample, they had a pH of 7.60 and 7.40 respectively. In contrast, the 'Flour, NaCl, $CaCO_3$' and 'Flour, NaCl' samples had the highest concentration of chaenorpine and both samples had a pH of 6.28 which was the lowest observed among the samples. This finding was of importance as it indicated that the relationship of pH scales up to production level processing from the designed SETP, an important link for food producers. In order to further understand this relationship a study that examined the pH of the systems independent from ingredients was designed.

Two additional products were formulated in order to lend insight into how pH and other ingredients impact the concentration of chaenorpine in extruded corn puffs. A 0.15% citric acid treatment resulted in a pH of 5.7. Furthermore, a treatment of 0.75% citric acid and 1% TSP resulted in a pH of 5.6 and was selected in order determine if pH and not TSP was responsible for the mitigation of chaenorpine in the final product. These two samples, plus those shown in Fig. 3.5 were plotted extruded product pH by the chaenorpine product concentration (shown in Figure 3.6). The negative correlation between the product pH and the concentration of chaenorpine in product was evident. Samples with a lower pH were found to contain a higher amount of chaenorpine than the systems that have basic pH. For example, the puffs produced with added citric acid had 0.60 mg/g of chaenorpine while puffs produced with TSP had 0.26 mg/g of chaenorpine. This decrease in chaenorpine concentration is over two-fold and affords insight on the development of bitterness mitigation strategies and improvement of overall acceptability.

Given the observed influence of product pH on the resultant concentration of chaenorpine in extruded puff products, the impact of flour storage was further evaluated. Previous work in corn flour reported that there was a decreased in overall liking by a consumer panel and increased bitterness with aged flour that was accompanied by a decrease in pH of the flour (134). Consequently, a shelf life study attempted to examine if an observed pH reduction during flour storage could lead to increased chaenorpine and thus bitterness perception.

The shelf life study consisted of raw flour that was stored at 25 and 35 °C for 1, 2, and 3 months. The flour was then subjected to SETP and the chaenorpine analyzed. Over

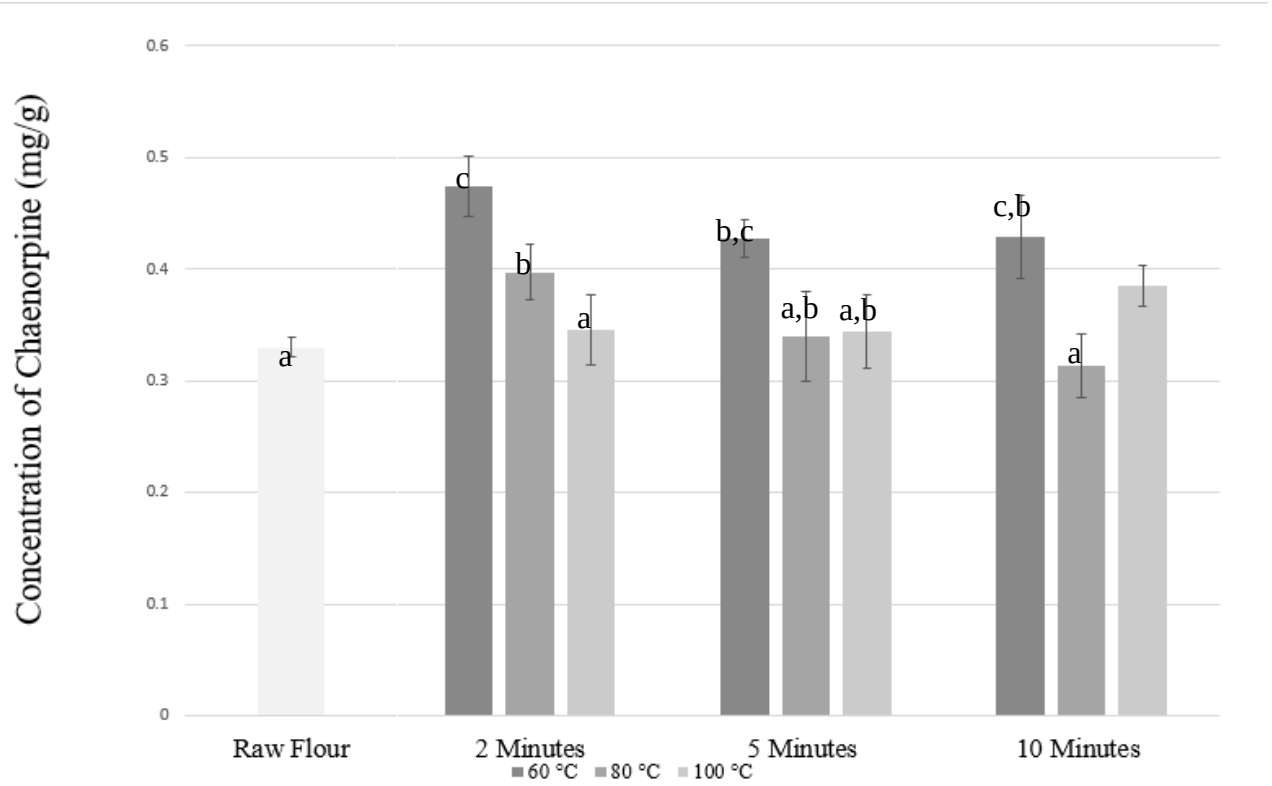
the course of the study there was no significant change on the pH of the system. This observation could be due to prior storage of the flour as it was not freshly milled but rather obtained commercially. Another potential confounding variable is the breed and growing conditions of the corn used for the previous studies. Additionally, the study showed that raw flour stored for up to 3 month did not show significant increase in chaenorpine. In other words, chaenorpine concentration did not change during storage alone. There was however an insignificant decreasing trend in the amount of chaenorpine in SETP samples. The findings of this portion of the study indicate that there was limited impact on the chaenorpine content of raw flour given our study parameters.

As a final part of this study, a sensory study was conducted to determine if the concentration changes of chaenorpine resulted in a perceived change in bitterness of the extruded corn puffs. Panelists ranked the formulations of perceived bitterness of each sample compared to known intensity standards. As seen in Table 3.2, the 'Flour, NaCl' or the 'Flour, NaCl, CaCO3' corn puff samples were perceived to have a statistically higher bitterness intensity. Alternatively, the 'Flour, TSP' sample had the lowest perceived bitterness and the highest pH (7.60). Furthermore, in Figure 3.9 a scatter plot of the concentration of chaenorpine all the puffed samples versus the perceived bitterness intensity, and demonstrated a positive linear relationship. This result is of particular importance as it correlates the observed analytical data to the sensory experience. The sensory study supported the analytical findings regarding the impact of pH on the concentration of chaenorpine and further indicated the importance of that formulation parameter in the overall bitterness perception of extruded corn systems.

Conclusions

The goal of this work was to determine if there are factors in processing that can influence chaenorpine, a key bitter compound in extruded whole grain corn puffs. Over the course of the study a wide array of variables were studied including, pre-heat treatment of the flour, ingredient modification/product pH, and flour storage. Results indicated that pH had a significant impact on the content of chaenorpine. It was shown through analytical quantitation that when pH of the system is higher the chaenorpine concentration is lower. The relationship of pH and quantitative amount of chaenorpine was demonstrated in both SETP benchtop processing and pilot scale processing and the negative correlation was observed for the total content observed. Sensory analysis results supported the analytical data and demonstrated that WGP with higher pH had a lower perceived bitterness compared to puffs with a lower pH. The study shows that pH control through processing parameters or product formulation can provide a feasible solution for flavor improvement of whole grain products and overall acceptability.

TABLES AND FIGURES



b

Figure 3.1: Comparison of pre-processing heat treatments on flour effect on chaenorpine content. Samples were heated at 60 (■), 80 (■) or 100 (■) °C for 2, 5 or 10min. Error bars denote the coefficient of variance of the samples (n = 3 and α=0.05). Samples with the same letters denote no statistical difference.

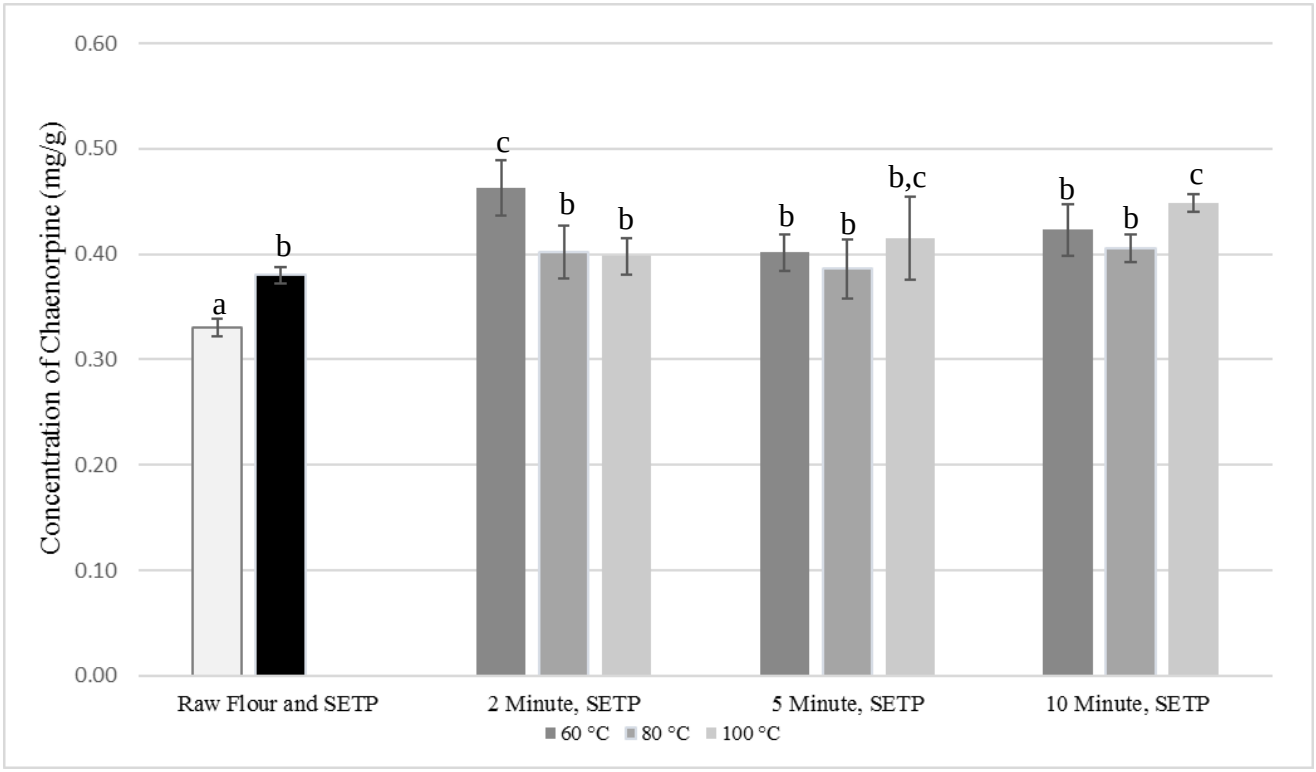


Figure 3.2: Comparison of pre-processing heat treatments with SETP samples chaenorpine concentration. Samples were heated at 60 (■), 80 (■) or 100 (■) °C for 2, 5 or 10min. Error bars denote the coefficient of variance of the samples (n = 3 and α=0.05). Samples with the same letters denote no statistical difference.

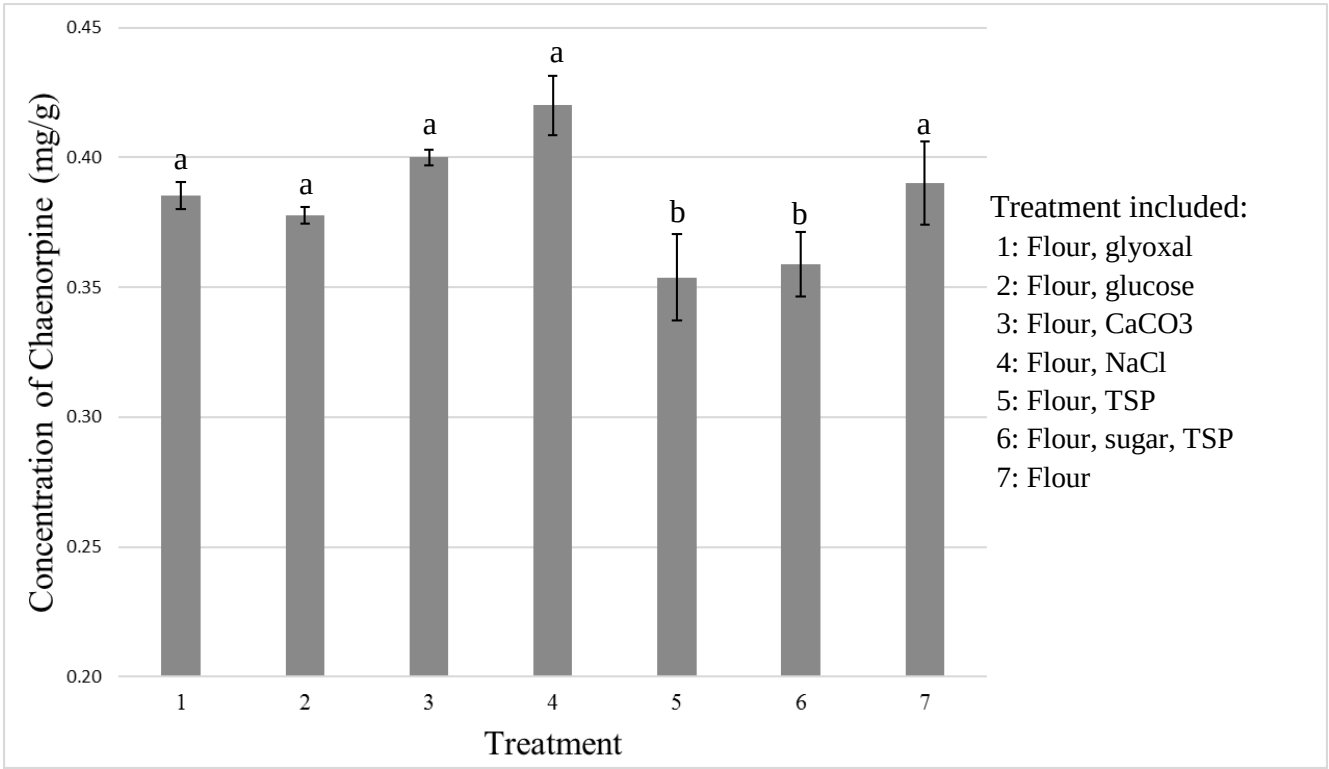


Figure 3.3: [DP5]Comparison of ingredients and processing aids impact on the concentration of chaenorpine in a SETP processing system. Samples underwent SETP [DP6]processing. Error bars denote the coefficient of variance of the samples (n = 3 and α=0.05). Samples with the same letters denote no statistical difference.

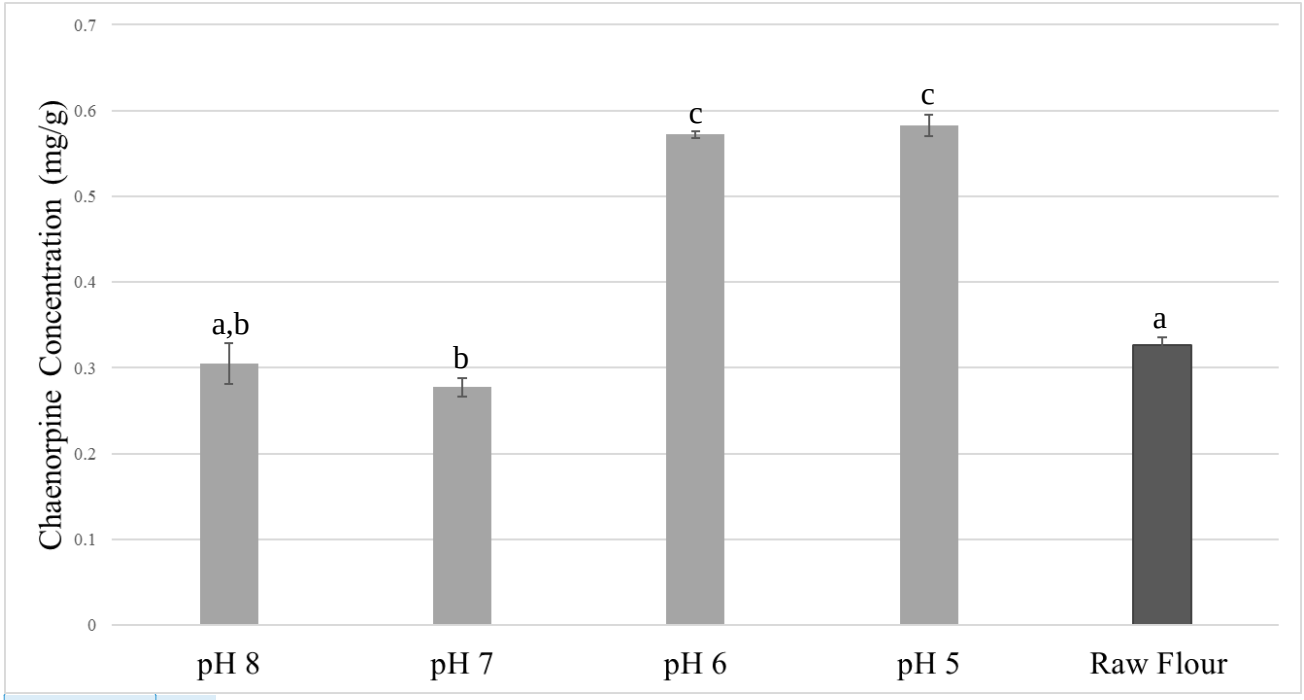


Figure 3.4: [DP7] Comparison of chaenorpine content in SETP samples with adjusted pH( ), Raw Flour denoted as) Error bars denote the coefficient of variance of the samples (n = 3 and α=0.05). Samples with the same letters denote no statistical difference.

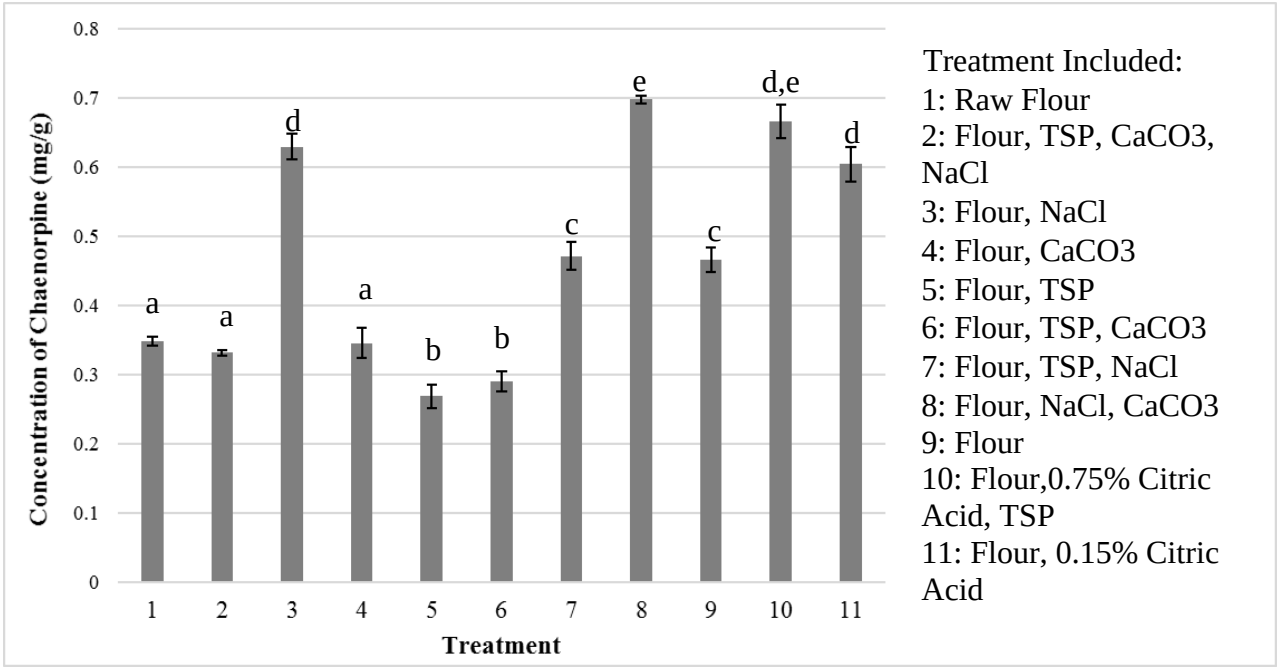


Figure 3.5: Comparison of product formulation chaenorpine concentration in samples made on a pilot plant scale extrusion. Samples were formulated with 1% Salt, 1% TSP, and or 1% $CaCO_3$. Error bars denote the coefficient of variance of the samples (n = 3 and $\alpha=0.05$). Samples with the same letters denote no statistical difference.

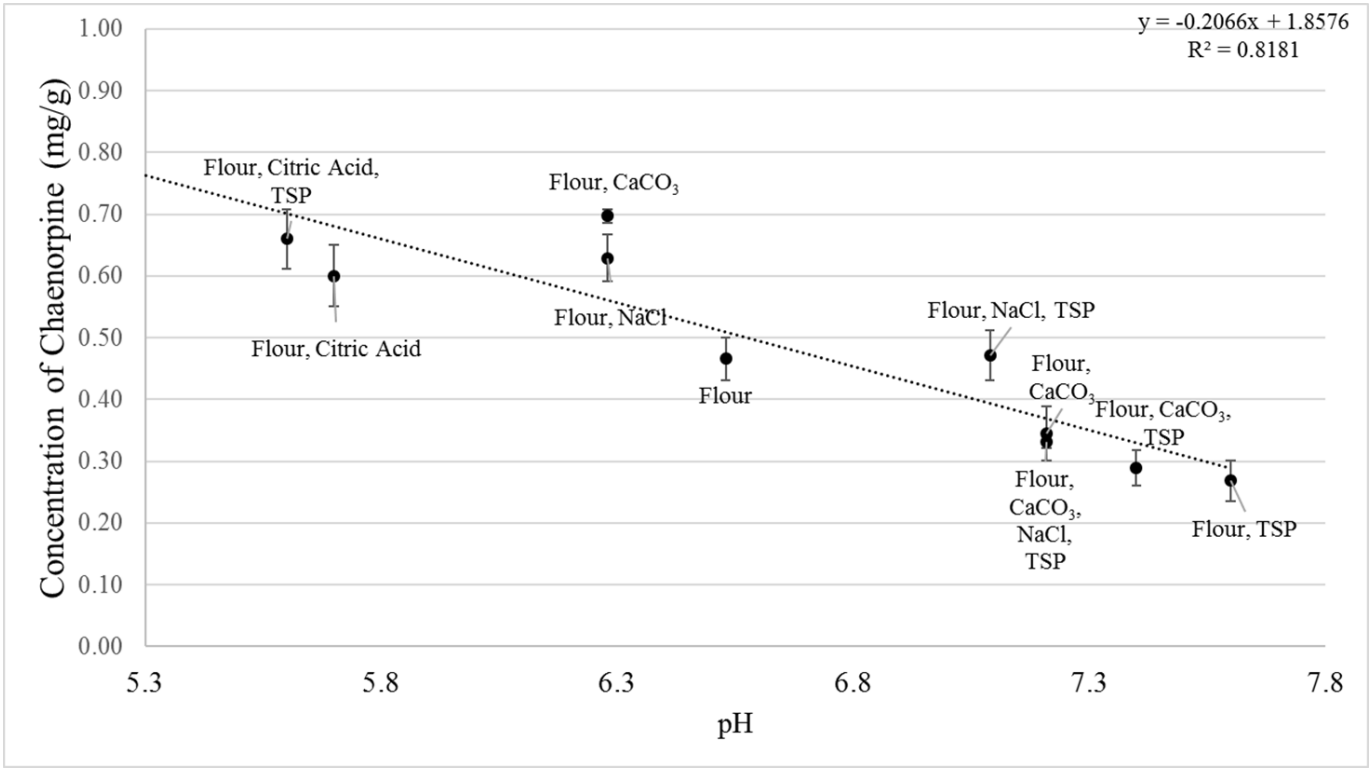


Figure 3.6: Extruded corn puff chaenorpine concentration versus product pH. Error bars denote the coefficient of variance of the samples (n = 3 and $\alpha=0.05$). Samples with the same letters denote no statistical difference.

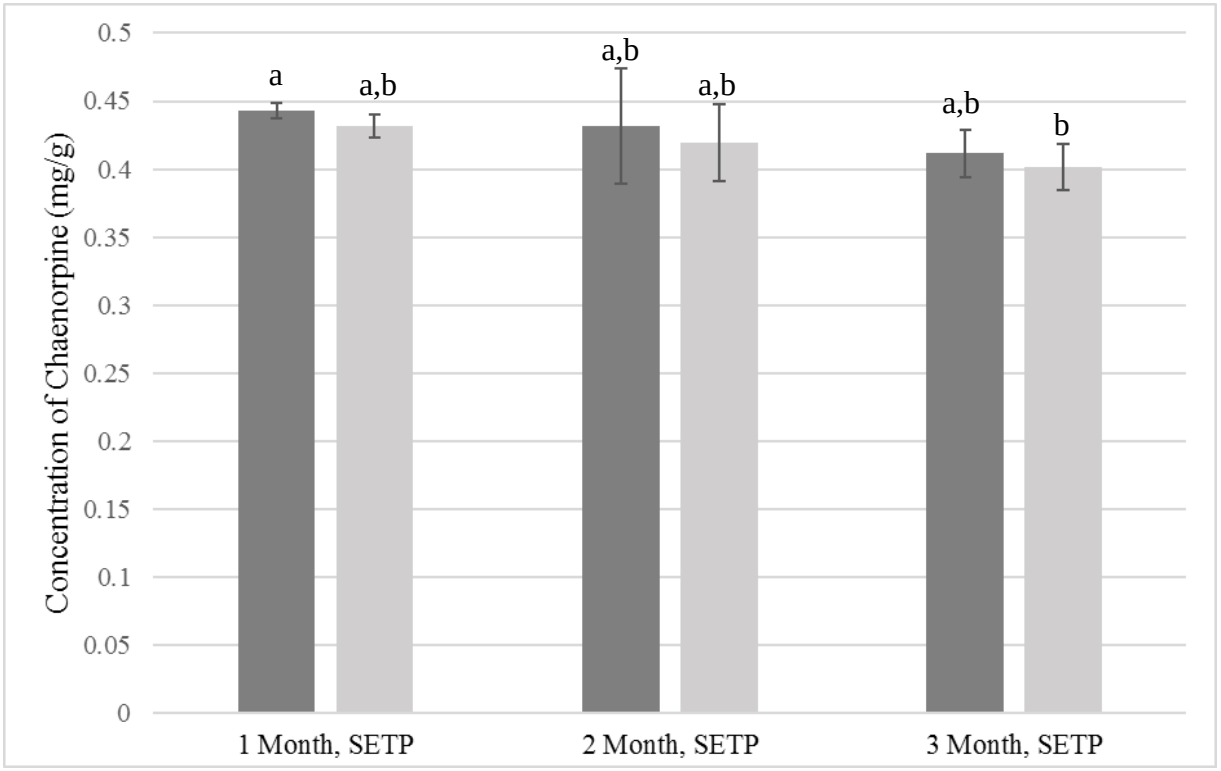


Figure 3.7: Comparison of flour age treatment and chaenorpine concentration following SETP. Flour samples stored for 1, 2 and 3 months at 25 °C( ) and 35 °C( ). Error bars denote the coefficient of variance of the samples (n = 3 and α=0.05). Samples with the same letters denote no statistical difference. (n = 3 and α = 0.05).

Table 3.1 Average intensity bitterness ratings of corn puffs made with different formulations.

Sample	Mean Bitterness Intensity	Group
Flour, NaCl	1.31	a
Flour, CaCO3,NaCl	1.25	a
Flour, TSP, NaCl	0.90	b
Flour	0.90	b
Flour, CaCO3	0.87	b
Flour, CaCO3, NaCl, TSP	0.76	b
Flour, CaCO3, TSP	0.59	b
Flour, TSP	0.57	b

Panel of 12 (8 female and 4 male, ages 21- 45). Different letters indicate a significant difference, statistical significance based on Tukey's LSD = 0.35 (significance level $p < 0.05$)

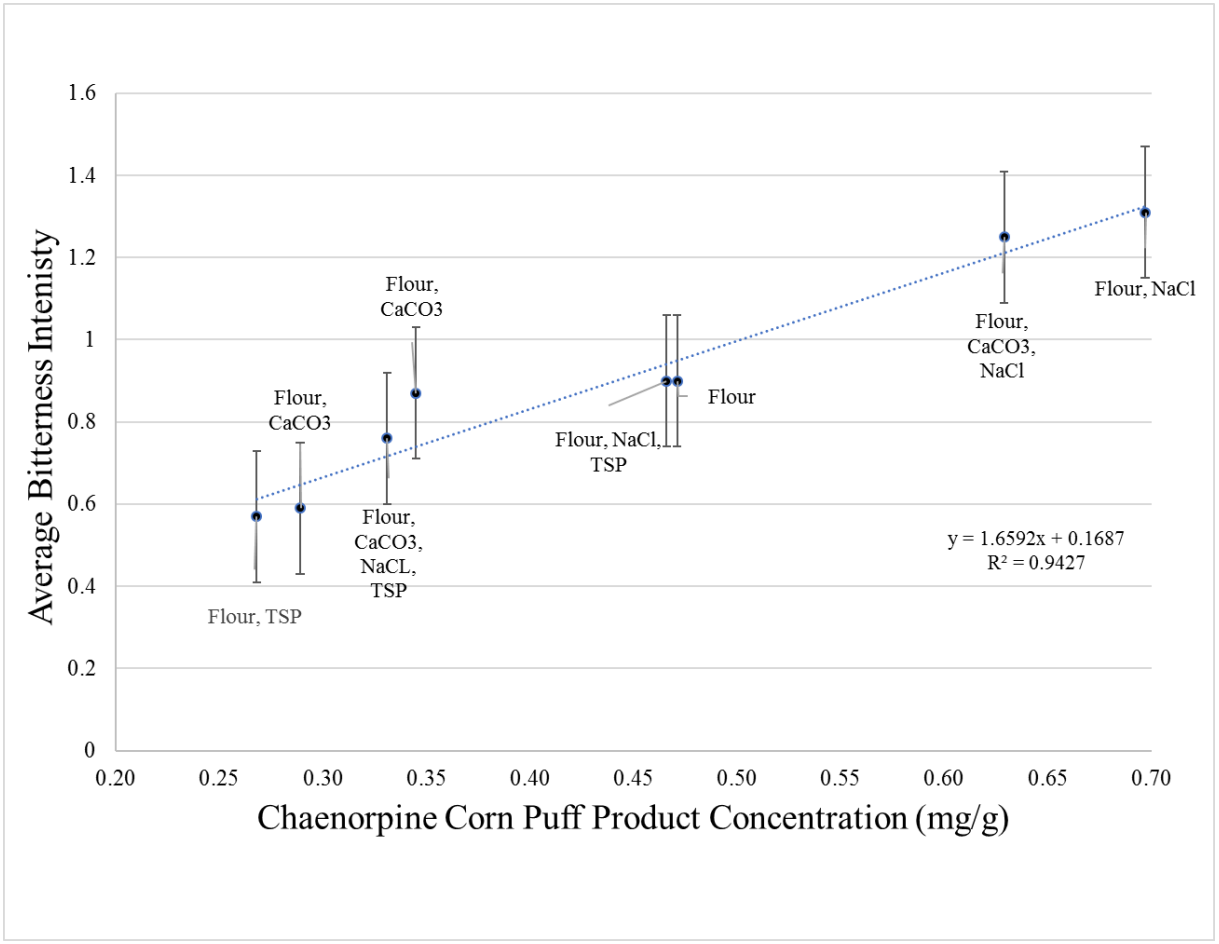


Figure 3.8: Relationship of chaenorpine corn puff concentration and the perceived bitterness intensity. Error bars denote Fisher's LSD = 0.35 (significance level $p < 0.05$)

Chapter 4: Suggested Future Work

The results presented here on aroma and the factors that influence the chaenorpine content of extruded corn puffs suggest there are further areas of research in 1) understanding how flavor is influenced in extruded systems by specific amino acid content, 2) a deeper exploration of how chaenorpine interacts with the starch matrix of corn. 3) full reaction mapping of chaenorpine to understand how the compound breaks down in an extruded system.

First the a deepened understanding of how amino acids influence the aroma profile of extruded corn systems. As seen in Table 2.2 the only compound that was higher in the RFP same was 2,5-dimethylpyrazine. In an effort to deepen the understanding of reaction pathways that occur in an extruder determining how individual components react and the flavor outcomes that they contribute to would be a step toward understanding how to design processing or tailor formulation for specific flavor outcomes.

Second, a wide variety of factors were examined for their impact on chaenorpine concentration in extruded corn products. However, despite the correlation between pH and the reduction a knowledge gap still exists in how chaenorpine is incorporated in the ingredient. Understanding how the compound is incorporated into the corn flour could deepen the understanding of how the compound is liberated or produced during food processing.

Third, as seen above there is compelling evidence that HCAs interact with the Maillard reaction through the formation of addicts. These adducts have flavor impacts in UHT milk and in whole grain bread. It is feasible that chaenorpine might also form

similar adducts with Maillard intermediates that were not examined in this study. By understanding how chaenorpine interacts with Maillard intermediates or precursors a reaction method for bitter marker detection or reaction pathway for degradation could be designed. Like understanding how amino acids influence the formation of aroma compounds understanding how Maillard precursors interact with chaenorpine could enable producers to develop reaction methods to mitigate chaenorpine in corn products.

References

1. Albertson, A.M., Reicks, M., Joshi, N. & Gugger, C. K. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001-2012. Nutr. J.15,8 (2016).

2. Shallenberger, R. S., Acree, T.E. Chemical structure of compounds and their sweet and bitter taste. In Taste (pp 221–277). Springer Berlin Heidelberg,1971

3. Classen, C., Howes, D., Synnott, A. Aroma: the cultural history of smell. Taylor & Francis. (1994)

4. Sorensen, LB., Moller, P., Martens, M., Raban, A.. Effect of Sensory Perception of Foods on Appetite and Food Intake: A Review of Studies on Humans. International Journal of Obesity (2003) 27, 1152–1166. doi:10.1038/sj.ijo.0802391

5. Slavin, J. (2004). Whole grains and human health. Nutrition Research Reviews, 17(1), 99–110. http://doi.org/10.1079/NRR200374

6. Williams, P. (2014) The Benefits of Breakfast Cereal Consumption: A Systematic Review of the Evidence Base. American Society for Nutrition. Adv. Nutr. 5: 636S–673S

7. McKeown, N. M., Meigs, J. B., Liu, S., Wilson, P. W. F., & Jacques, P. F. (2002). Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. The American Journal of Clinical Nutrition, 76(2), 390–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12145012

8. Jenkins, D. J., Wesson, V., Wolever, T. M., Jenkins, a L., Kalmusky, J., Guidici, S., … Wong, G. S. (1988). Whole meal versus wholegrain breads: proportion of whole or cracked grain and the glycemic response. BMJ (Clinical Research Ed.), 297(6654), 958–960. http://doi.org/10.1136/bmj.297.6654.958

9. Terry, P., Laguerre, J., Ye, W., Walk, a, & Myron, O. (2001). Inverse association between intake of cereal fiber and risk of gastric cardia cancer. Gastroenterology, 120(2), 387–391. http://doi.org/10.1053/gast.2001.21171

10. Kantor, L. S., Varian, J. N., Almshouse, J. E., Putnam, J. J., & Lin, B. (2001). The Dietary Guidelines : Surveillance Issues and Research Needs Choose a Variety of Grains Daily , Especially Whole Grains : A Challenge for Consumers 1, (3), 473–486.

11. Aksum, C. M., Jacobs, D. R., Nicodemus, K., & Folsom, A. R. (2002). Dietary risk factors for upper autodigestive tract cancers. International Journal of Cancer, 99(2), 267–272. http://doi.org/10.1002/ijc.10341

12. Anderson, J. (2000). Whole grains and coronary heart disease. The American Journal of Clinical Nutrition, 71(4), 1009.

13. U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary guidelines for Americans. 8th Edition. 2015-2020, Available at http://health.gov/dietaryguidelines/2015/guidelines/

14. EPA, (2012) Agriculture 101: Crop Production, http://www.epa.gov/agriculture/ag101/printcrop.html, October 23, 2012

15. Birkett, A. M., & Cho, S. S. (2013). Cereal fiber and health: Current knowledge. Cereal Foods World, 58(6), 309–313. http://doi.org/10.1094/CFW-58-6-0309

16. Mintel's Breakfast cereals-US. August 2013

17. Marchant, J., Reuban, B., and Alcock, J. (2009) Bread: A Slice of History. The History Press, Print Copy

18. Severson, K. (2016). A Short History of Cereal. Retrieved from https://www.nytimes.com/interactive/2016/02/22/dining/history-of-cereal.html

19. Jaya, S. (2015). How Cereal Became the Quintessential American Breakfast. Retrieved from http://www.seriouseats.com/2015/03/history-of-breakfast-cereal-mascots.html

20. Frame, N. D. (Ed.). (1994). The Technology of Extrusion Cooking (1st ed.). Peterborough: Springer Science and Business Media Dordrecht.

21. Miller, R. A., Jeong, J., Maningat, C. C. Effect of RS4 Resistant Starch on Extruded Ready-to-Eat (RTE) Breakfast Cereal Quality. Cereal Chem. 584–588 (2006)

22. Nair, M., Shi, Z., Karwe Mukund, V., Ho, C.-T., Daun, H. Collection and Characterization of Volatile Compounds Released at the Die during Twin Screw Extrusion of Corn Flour. Therm. Gener. Flavors 334–347 (1993).

23. Bredie, W. L. P., Mottram, D. S., & Guy, R. C. E. (1998). Aroma Volatiles Generated during Extrusion Cooking of Maize Flour, 8561(97).

24. Villota, R.,Hawkes, J. . Flavoring in extrusion. An overview. Therm. Gener. Flavors Mail. Microwave, Extrus. Process 280–296 (1994).

25. Dyck, V., Decker, E., Elias, R., McClements, D. (2010) Oxidation in foods and beverages and antioxidant applications. Woodhead Publishing,

26. Ho, C., Chen, Q., State, T., & Brunswick, N. (1994). Lipids in Food Flavors, 2–14.

27. Martins, S. I. F. S., Jongen, W. M. F. & Boekel, M. A. J. S. Van. A review of Maillard reaction in food and implications to kinetic modelling. Trends in Food Science & Technology 11, 364–373 (2001)

28. Mottram, D. S., & Elmore, J. S. (2010). Control of the Maillard Reaction during the cooking of food. Chapter 14: In Controlling Maillard Pathways to Generate Flavors.

29. Van Ba, H., Hwang, I., Jeong, D., & Touseef, A. (2012). Principle of Meat Aroma Flavors and Future Prospect. Latest Research into Quality Control, 145–176. http://doi.org/37707

30. Hofmann, T. Taste-active Maillard reaction products: the 'tasty' world of nonvolatile Maillard reaction products. Ann. N. Y. Acad. Sci. 1043, 20–29 (2005).

31. Clifford MN (1999) Chlorogenic acids and other cinnamates— nature, occurrence, dietary burden, absorption and metabolism. J Sci Food Agric 80:1033–1043

32. Li L, Shewry PR, Ward JL (2008) Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. J Agric Food Chem. 56:9732–9739

33. Buttery R, Stern D, Ling L (1994) Studies on flavor volatiles of some sweet corn products. J Agric Food Chem. 42:791–795

34. Schieberle P (1991) Primary odorants in popcorn. J Agric Food Chem. 39:1141–1144

35. Buttery R, Ling L (1995) Volatile flavor components of corn tortillas and related products. J Agric Food Chem. 43: 1878–1882

36. Jiang, D. & Peterson, D (2010) Role of hydroxycinnamic acids in food flavor: a brief review. Phyto-chemistry Review, 2010(9): 187-193

37. Heinio RL, Liukkonen KH, Myllyma O, Pihlava JM, Adlercreutz H, Heinonen SM, Poutanen K (2008) Quantities of phenolic compounds and their impacts on the perceived flavor attributes of rye grain. J Cereal Sci 566–575

38. Chandrashekar, J., Hoon, M. A., Ryba, N. J. P. & Zuker, C. S., The receptors and cells for mammalian taste. Nature, 2006; Vol. 444, pp 288-294.

39. Nelson, G. et al. An Amino-Acid Taste Receptor. Nature 416, 199–202 (2002)

40. Lindemann, B. Receptors and transduction in taste. Nature 413, 219–225 (2001)

41. Damodaran, S., Parkin, K. L., & Fennema, O. R. (2008). *Fennema's food chemistry*. Boca Raton: CRC Press/Taylor & Francis.

42. Solms, J., & In-, S. F. (1967). The Taste of Amino Acids, Peptides, and Proteins.

43. Hofmann, T., Ho, C. & Pickenhagen, W. Challenges in Taste Research : Present Knowledge and Future Implications. ACS Symposium series American Chemical Society(USA). American Chemical Society (2004)

44. Drewnowski, A., Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: a review. Am J Clinical Nutr 72, 1424–1435 (2000)

45. Saroli, A. Structure-activity relationship of a bitter compound: Denatonium chloride. Naturwissenschaften 71, 428–429 (1984)

46. Wiener, A., Shudler, M., Levit, A. & Niv, M. Y. Bitter DB: A database of bitter compounds. Nucleic Acids Res. 40, 413–419 (2012)

47. Zhang, L., (2016) Impact of Thermal Processing on Taste Development in Food, A dissertation submitted to the faculty of the University of Minnesota

48. Horowitz, R. M. & Gentili, B. Taste and Structure in Phenolic Glycosides. J. Agric. Food Chem. 17, 696–700 (1969).

49. Deibler, K., Delwiche, J (2003). Handbook of Flavor Characterization: Sensory Analysis, Chemistry, and Physiology, Hardcover. CRC Press. ISBN: 9780824747039

50. Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices, 237–241.

51. Delahunty, C. M., Eyres, G., & Dufour, J. (2006). Review Gas chromatography-olfactometry, 2107–2125. http://doi.org/10.1002/jssc.200500509

52. Acree, T., Heinrich, A., (2004) Flavor Net, www.flavornet.org, June 25, 2004

53. Hofmann, T. Taste-active Maillard reaction products: the 'tasty' world of nonvolatile Maillard reaction products. Ann. N. Y. Acad. Sci. 1043, 20–29 (2005).

54. Grosch, W. (1994). Determination of Potent Odorants in Foods by Aroma Extract Dilution Analysis (AEDA) and Calculation of Odor Activity Values (OAVs), 9(April).

55. G. Lampman, S. Kriz, J. Vyvyan (2008). Introduction to Spectroscopy 4th Edition, Brooks Cole Publishing

56. Potts, D. (2016). Factors Affecting Flavor Quality of Bovine Milk and Dairy Products, Ph. D. Dissertation University of Minnesota

57. Lawless, H. T., & Heymann, H. (2010). Sensory Evaluation of Food: Principles and Practices Springer Science & Business Media. Retrieved from https://books.google.com/books?id=yrLfrVgU6CsC&pgis=1

58. Stark, T.,Hofmann, T. Structures, sensory activity, and dose/response functions of 2,5-diketopiperazines in roasted cocoa nibs (Theobroma cacao). J. Agric. Food Chem. 53, 7222–7231 (2005).

59. Stark, T., Bareuther, S. & Hofmann, T. Molecular definition of the taste of roasted cocoa nibs (Theobroma cacao) by means of quantitative studies and sensory experiments. J. Agric. Food Chem. 54, 5530–5539 (2006).

60. Stark, T.,Hofmann, T. Structures, sensory activity, and dose/response functions of 2,5-diketopiperazines in roasted cocoa nibs (Theobroma cacao). J. Agric. Food Chem. 53, 7222–7231 (2005).

61. Ottinger, H., Soldo, T. & Hofmann, T. Discovery and structure determination of a novel Maillard-derived sweetness enhancer by application of the comparative taste dilution analysis (cTDA). J. Agric. Food Chem. 51, 1035–1041 (2003)

62. Ottinger, H. & Hofmann, T. Identification of the Taste Enhancer Alapyridine in Beef Broth and Evaluation of Its Sensory Impact by Taste Reconstitution Experiments. J. Agric. Food Chem. 51, 6791–6796 (2003).

63. Stark, T., Bareuther, S. & Hofmann, T. Sensory-Guided Decomposition of Roasted Cocoa Nibs (Theobroma cacao) and Structure Determination of Taste Active Polyphenols. J. Agric. Food Chem. 53, 5407–5418 (2005).

64. Frank, O., Jezussek, M. & Hofmann, T. Sensory activity, chemical structure, and synthesis of Maillard generated bitter-tasting 1-oxo-2,3-dihydro-1H-indolizinium-6-oleates. J. Agric. Food Chem. 51, 2693-9 (2003)

65. Jiang, D. & Peterson, D. G. Identification of bitter compounds in whole wheat bread. Food Chem. 141, 1345–1353 (2013).

66. Liu, X., Jiang, D. & Peterson, D. G. Identification of bitter peptides in whey protein hydrolysate. J. Agric. Food Chem. 62, 5719–25 (2014).

67. Zhang, L & Peterson D.

68. Usol, L., & Son, A. T. (n.d.). C : Food Chemistry and Toxicology Use of Mild Heat Pre-treatment for Quality Retention of Fresh-cut Cantaloupe Melon.

69. Moskowitz, M. R., Bin, Q., Elias, R. J., & Peterson, D. G. (2012). Influence of endogenous ferulic acid in whole wheat flour on bread crust aroma. Journal of Agricultural and Food Chemistry, 60(45), 11245–52. http://doi.org/10.1021/jf303750y

70. Kokkinidou, S., & Peterson, D. G. (2014). Control of Maillard-type off-flavor development in ultrahigh-temperature-processed bovine milk by phenolic chemistry. Journal of Agricultural and Food Chemistry, 62(32), 8023–33. http://doi.org/10.1021/jf501919y

71. Colahan-Sederstrom, P. M.; Peterson, D. G. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. J. Agric. Food Chem. 2005, 53, 398− 402

72. Totlani, V.M. and Peterson, D.G. 2007. Influence of Epicatechin Reactions on the Mechanisms of Maillard Product Formation in Low Moisture Model Systems. J. Ag. Food Chem. 55: 414-420.http://pubs.acs.org/doi/full/10.1021/jf0617521

73. Shobha, D., Dileep, H. V, & Sreeramasetty, T. A. (2014). Storage influence on the functional , sensory and keeping quality of quality protein maize flour, 51(November), 3154–3162. http://doi.org/10.1007/s13197-012-0788-7

74. Bin, Q., Jiang, D., Cho, I. H., & Peterson, D. G. (2012). Chemical markers for bitterness in wheat bread. Flavor and Fragrance Journal, 27(6), 454–458. http://doi.org/10.1002/ffj.3124

Chapter 2 References

75. Acree, T., Heinrich, A., (2004) Flavor Net, www.flavornet.org, June 25, 2004

76. Andersson, R., Kamal-eldin, A., Mustafa, A., & Per, A. (2007). Food Chemistry Analysis of free amino acids in cereal products, *105*, 317–324. http://doi.org/10.1016/j.foodchem.2006.11.044

77. Anderson, J. (2000). Whole grains and coronary heart disease. *The American Journal of Clinical Nutrition*, *71*(4), 1009.

78. Birkett, A. M., & Cho, S. S. (2013). Cereal fiber and health: Current knowledge. *Cereal Foods World*, *58*(6), 309–313. http://doi.org/10.1094/CFW-58-6-0309

79. Bredie, W. L. P., Mottram, D. S., & Guy, R. C. E. (1998). Aroma Volatiles Generated during Extrusion Cooking of Maize Flour, *8561*(97).

80. Chatenoud, L., La Vecchia, C., Franceschi, S., Tavani, a, Jacobs, D. R., Parpinel, M. T., … Negri, E. (1999). Refined-cereal intake and risk of selected cancers in italy. *The American Journal of Clinical Nutrition*, *70*(6), 1107–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10584057

81. Colahan-Sederstrom, P. M., & Peterson, D. G. (2005). Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via

epicatechin addition. Journal of Agricultural and Food Chemistry, 53(2), 398–402. http://doi.org/10.1021/jf0487248Chatenoud,

82. L., La Vecchia, C., Franceschi, S., Tavani, a, Jacobs, D. R., Parpinel, M. T., … Negri, E. (1999). Refined-cereal intake and risk of selected cancers in italy. *The American Journal of Clinical Nutrition*, *70*(6), 1107–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10584057

83. Deibler, K., Delwiche, J (2003). Handbook of Flavor Characterization: Sensory Analysis, Chemistry, and Physiology, Hardcover. CRC Press. ISBN: 9780824747039

84. Djilas, S. M., & Milić, B. L. (2005). Naturally Occurring Phenolic Compounds as Inhibitors of Free Radical Formation in the Maillard Reaction. In Maillard Reactions in Chemistry, Food and Health (pp. 75–81). Elsevier. http://doi.org/10.1533/9781845698393.2.75

85. Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices, 237–241.

86. EPA, (2012) Agriculture 101: Crop Production, http://www.epa.gov/agriculture/ag101/printcrop.html, October 23, 2012

87. Flight, I., & Clifton, P. (2006). Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *European Journal of Clinical Nutrition*, *60*(10), 1145–59. http://doi.org/10.1038/sj.ejcn.1602435

88. Fors, S. (1983). Products and Related Compounds.

89. Grosch, W. (1994). Determination of Potent Odourants in Foods by Aroma Extract Dilution Analysis (AEDA) and Calculation of Odour Activity Values (OAVs), *9*(April).

90. Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chemical Senses*, *26*(5), 533–45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11418500

91. Gwirtz, J. A., & Garcia-casal, M. N. (2014). Processing maize flour and corn meal food products, *1312*, 66–75. http://doi.org/10.1111/nyas.12299

92. Delgado, RM., Hidalgo, FJ., Zamora, R. Antagonism between lipid-derived reactive carbonyls and phenolic compounds in the Strecker degradation of

amino acids (2016) J. Food Chemistry194:1143-8. doi: 10.1016/j.foodchem.2015.07.126

94. Ho, C., Chen, Q., State, T., & Brunswick, N. (1994). Lipids in Food Flavors, 2–14.

94. Jenkins, D. J., Wesson, V., Wolever, T. M., Jenkins, a L., Kalmusky, J., Guidici, S., … Wong, G. S. (1988). Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *BMJ (Clinical Research Ed.)*, *297*(6654), 958–960. http://doi.org/10.1136/bmj.297.6654.958

95. Jiang, D. & Peterson, D (2010) Role of hydroxycinnamic acids in food flavor: a brief review. Phyochemisty Review , 2010(9): 187-193

96. Kantor, L. S., Variyam, J. N., Allshouse, J. E., Putnam, J. J., & Lin, B. (2001). The Dietary Guidelines : Surveillance Issues and Research Needs Choose a Variety of Grains Daily , Especially Whole Grains : A Challenge for Consumers 1, (3), 473–486.

97. Kasum, C. M., Jacobs, D. R., Nicodemus, K., & Folsom, A. R. (2002). Dietary risk factors for upper aerodigestive tract cancers. *International Journal of Cancer*, *99*(2), 267–272. http://doi.org/10.1002/ijc.10341

98. Kokkinidou, S., & Peterson, D. G. (2014). Control of maillard-type off-flavor development in ultrahigh-temperature-processed bovine milk by phenolic chemistry. Journal of Agricultural and Food Chemistry, 62(32), 8023–33. http://doi.org/10.1021/jf501919yKasum, C. M., Jacobs, D. R., Nicodemus, K., & Folsom, A. R. (2002). Dietary risk factors for upper aerodigestive tract cancers. *International Journal of Cancer*, *99*(2), 267–272. http://doi.org/10.1002/ijc.10341

99. Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food: Principles and Practices*. Springer Science & Business Media. Retrieved from https://books.google.com/books?id=yrLfrVgU6CsC&pgis=1

100. McKeown, N. M., Meigs, J. B., Liu, S., Wilson, P. W. F., & Jacques, P. F. (2002). Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *The American Journal of Clinical Nutrition*, *76*(2), 390–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12145012

101. Moskowitz, M., Bing, Q., Elias, R. and Peterson, D.G. 2012. Influence of endogenous ferulic acid in whole wheat flour on bread crust aroma. J. Agric. Food Chem. 60: 11245-11252

102. Mottram, D. S., & Elmore, J. S. (2010). Control of the Maillard Reaction during the.

103. Mintel's Breakfast cereals-US. August 2013

104.Nair, M., Shi, Z., Karwe Mukund, V., Ho, C.-T., Daun, H. Collection and Characterization of Volatile Compounds Released at the Die during Twin Screw Extrusion of Corn Flour. Therm. Gener. Flavors 334–347 (1993).

105. Peleg, H., Naim, M., Rouseff, R. L., & Zehavi, U. (1991). Distribution of Bound and Free Phenolic-Acids in Oranges (Citrus-Sinensis) and Grapefruits (Citrus-Paradisi). *Journal of the Science of Food and Agriculture*, *57*(3), 417–426. Retrieved from <Go to ISI>://A1991GX92400011

106. Potts, D. (2016). Factors Affecting Flavor Quality of Bovine Milk and Dairy Products, Ph. D. Dissertation University of Minnesota

107. Roe, M.A., Faulks, R.M., Belsten, J.L. (1990). Role of Reducing Sugars and Amino Acids in Fry Color of Chips from Potatoes Grown under Different Nitrogen Regimes. J. of Science of Food and Agriculture. 52(2):207-214

108. Slavin, J. (2004). Whole grains and human health. *Nutrition Research Reviews*, *17*(1), 99–110. http://doi.org/10.1079/NRR200374

109. Shah, A. R., Shah, R. K., & Madamwar, D. (2006). Improvement of the quality of whole wheat bread by supplementation of xylanase from Aspergillus foetidus. *Bioresource Technology*, *97*(16), 2047–2053. http://doi.org/10.1016/j.biortech.2005.10.006

110. Snow, G.M.: Using the principles of social marketing to explore adolescents' perceptions and preferences related to food and nutrition education. Unpublished master's thesis, University of Nevada, Reno, 1999

111. Sobal J, Beckman L, Pham A, Croy M, Marquart L. Situational food choices: Social representations of where, when, and who consumes whole grain foods. Topics in Clinical Nutrition 2010;25(1):75-83.

112. Terry, P., Lagergren, J., Ye, W., Wolk, a, & Nyrén, O. (2001). Inverse association between intake of cereal fiber and risk of gastric cardia cancer. *Gastroenterology*, *120*(2), 387–391. http://doi.org/10.1053/gast.2001.21171

113. U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary guidelines for Americans. 8th Edition. 2015-2020, Available at http://health.gov/dietaryguidelines/2015/guidelines/ 136.

114. Kirchhoff, E.; Schieberle, P. Determination of key aroma compounds in the crumb of a three-stage sourdough rye breadby stable isotope dilution assays and sensory studies. J. Agric. Food Chem. 2001, 49, 4304-4311.

115. Leffingwell and Associates, Odor & Flavor Detection Thresholds in Water, http://www.leffingwell.com/odorthre.htm

116. Van Ba, H., Hwang, I., Jeong, D., & Touseef, A. (2012). Principle of Meat Aroma Flavors and Future Prospect. *Latest Research into Quality Control*, 145–176. http://doi.org/37707

117. Yaylayan, V. A. (1997). Classification of the Maillard reaction: A conceptual approach. *Trends in Food Science & Technology*, *8*(1), 13–18. http://doi.org/10.1016/S0924-2244(96)20013-5

Chapter 3 References

118. Adams, J. F., Rd, M. S., Rd, A. E., Adams, J. F., Rd, M. S., & Engstrom, A. (2017). Helping Consumers Achieve Recommended Intakes of Whole Grain Foods Helping Consumers Achieve Recommended Intakes of, 5724(April). http://doi.org/10.1080/07315724.2000.10718970\

119. Aune, D., & Chan, D. S. M. (2010). Dietary fiber, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies, 6617(November), 1–20. http://doi.org/10.1136/bmj.d6617

120. Boz H. (2015): Ferulic acid in cereals – a review. Czech J. Food Sci., 33: 1–7

121. Bin, Q., Jiang, D., Cho, I. H., & Peterson, D. G. (2012). Chemical markers for bitterness in wheat bread. Flavor and Fragrance Journal, 27(6), 454–458. http://doi.org/10.1002/ffj.3124

122. Bredie, W. L. P., Mottram, D. S., & Guy, R. C. E. (1998). Aroma Volatiles Generated during Extrusion Cooking of Maize Flour, 8561(97).

123. Buddrick, O., Jones, O. A. H., Cornell, H. J., & Small, D. M. (2014). The influence of fermentation processes and cereal grains in wholegrain bread on reducing phytate content. *Journal of Cereal Science*, *59*(1), 3–8. http://doi.org/10.1016/j.jcs.2013.11.006

124. Buttery, R. G., Ling, L. C., & Stern, D. J. (1997). Studies on Popcorn Aroma and Flavor Volatiles, 8561(96).

125. Coral, D.F., Pineda-Gomez P., Rosales-Rivera A., & Rodriguez-Garcia M. E. (2009). Determination of the Gelatinization Temperature of Starch Presented in Maize Flours Journal of Physics: Conference Series, 831(70). http://doi.org/10.1088/1742-6596/167/1/012057

126. Fellows, P. (2006). *Food Processing Technology, 3rd Edition*. Woodhead Publishing 2009.

127. Golstein, A., Komeine Kotokeni Mekondjo Nantanga, Seetheraman, K., Molecular Interactions in Starch-Water Systems: Effect of Increasing Starch Concentration. doi:10.1094/CCHEM-87-4-0370

128. Kokkinidou, S., & Peterson, D. G. (2014). Control of Maillard-type off-flavor development in ultrahigh-temperature-processed bovine milk by phenolic chemistry. Journal of Agricultural and Food Chemistry, 62(32), 8023–33. http://doi.org/10.1021/jf501919y

129. Miller, R. A., Jeong, J., & Maningat, C. C. (2011). Effect of RS4 Resistant Starch on Extruded Ready-to-Eat (RTE) Breakfast Cereal Quality. *Cereal Chemistry*, *88*, 584–588.

130. Mintel's Breakfast cereals-US. August 2013

131. Mottram, D. S., & Elmore, J. S. (2010). Control of the Maillard Reaction during the cooking of food. Chapter 14: In Controlling Maillard Pathways to Generate Flavors.

132. Ratnayake, W. S., & Jackson, D. S. (2006). Gelatinization and Solubility of Corn Starch during Heating in Excess Water : New Insights in Excess Water : New Insights. http://doi.org/10.1021/jf0529114

133. Sacchetti, G. (2004). Effects of extrusion temperature and feed composition on the functional , physical and sensory properties of chestnut and rice flour-based snack-like products, 37, 527–534. http://doi.org/10.1016/j.foodres.2003.11.009

134. Shobha, D., Dileep, H. V, & Sreeramasetty, T. A. (2014). Storage influence on the functional , sensory and keeping quality of quality protein maize flour, *51*(November), 3154–3162. http://doi.org/10.1007/s13197-012-0788-7

135. Sobal, J., PhD, MPH; Beckman, L., B., Anh-Tram Pham, BS; Croy, M., MS, R., & Marquart, L., PhD, R. (2010). Situational Food Choices Social Representations of Where, When, and Who Consumes Whole-Grain Foods. Influences on Food Choices, 25(1), 75–83.

136. Slavin, J. (2004). Whole grains and human health. Nutrition Research Reviews, 17(1), 99–110. http://doi.org/10.1079/NRR200374

137. Snow, G.M.: Using the principles of social marketing to explore adolescents' perceptions and preferences related to food and nutrition education. Unpublished master's thesis, University of Nevada, Reno, 1999

138. Svihus, B., Uhlen, A. K., & Harstad, O. M. (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review, 122, 303–320. http://doi.org/10.1016/j.anifeedsci.2005.02.025

139. U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary guidelines for Americans. 8th Edition. 2015-2020, Available at http://health.gov/dietaryguidelines/2015/guidelines/

140. Yongfeng, A., Jay-lin Jane, (2016) Macronutrients in Corn and Human Nutrition. Comprehensive Reviews in Food Science and Food Safety. Vol.15, 2016: 581-597. doi:10.1111/1541-4337.12192

141. Zhang, L., (2017) Characterization of Key Bitter Compounds in Extruded Corn Puffed Products (EDITING NOTE: PENDING PUBLICATION)